WO2006082412

Publication Title:

MULTIMERS OF HETEROCYCLIC COMPOUNDS AND THEIR USE

Abstract:

Abstract of WO2006082412

A compound of formula (I): M-L-M, wherein L is a linker and each M is independently a group of formula (II): is useful in therapy, e.g. of respiratory diseases. Data supplied from the esp@cenet database - Worldwide

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 August 2006 (10.08.2006)

PCT

(10) International Publication Number
WO 2006/082412 A2

(51) International Patent Classification:
C07D 239/22 (2006.01) *A61K 31/513* (2006.01)
C07D 401/12 (2006.01) *A61P 9/00* (2006.01)
C07D 403/14 (2006.01)

(21) International Application Number: PCT/GB2006/000361

(22) International Filing Date: 3 February 2006 (03.02.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0502258.7 3 February 2005 (03.02.2005) GB

(71) Applicant (*for all designated States except US*): ARGENTA DISCOVERY LTD [GB/GB]; 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **FINCH, Harry** [GB/GB]; Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR (GB). **EDWARDS, Christine** [GB/GB]; Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR (GB). **RAY, Nicholas, Charles** [GB/GB]; Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR (GB). **O'CONNOR, Elizabeth, Anne** [GB/GB]; Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR (GB). **FITZGERALD, Mary, F.** [GB/GB]; Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR (GB).

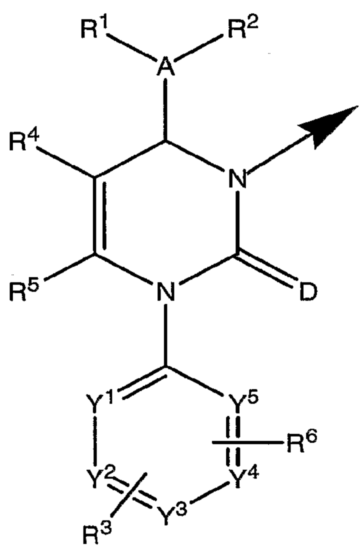
(74) Agent: GILL JENNINGS & EVERY LLP; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,

[Continued on next page]

(54) Title: MULTIMERS OF HETEROCYCLIC COMPOUNDS AND THEIR USE

M-L-M (I)



(II)

(57) Abstract: A compound of formula (I): M-L-M, wherein L is a linker and each M is independently a group of formula (II): is useful in therapy, e.g. of respiratory diseases.

WO 2006/082412 A2

LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

MULTIMERS OF HETEROCYCLIC COMPOUNDS AND THEIR USE

Field of the Invention

This invention relates to heterocyclic compounds and their use in therapy.

Background to the invention

Human neutrophil elastase is a 32 kDa serine proteinase found in the azurophilic granules of neutrophils. It has a role in the degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, Type III and Type IV collagens as well as elastin (Bieth, G. In *Regulation of Matrix accumulation*, Mecham, R. P. (Eds), Academic Press, NY, USA 1986, 217-306). HNE has long been considered to play an important role in homeostasis through repair and disposal of damaged tissues via degradation of the tissue structural proteins. It is also relevant in the defence against bacterial invasion by means of degradation of the bacterial body. In addition to its effects on matrix tissues, HNE has been implicated in the upregulation of IL-8 gene expression and also induces IL-8 release from the epithelial cells of the lung. In animal models of Chronic Obstructive Pulmonary Disease induced by tobacco smoke exposure both small molecule inhibitors and protein inhibitors of HNE inhibit the inflammatory response and the development of emphysema (Wright, J. L. et al. *Am. J. Respir. Crit. Care Med.* 2002, *166*, 954-960; Churg, A. et al. *Am. J. Respir. Crit. Care Med.* 2003, *168*, 199-207). Thus, HNE may play a role both in matrix destruction and in amplifying inflammatory responses in chronic respiratory diseases where neutrophil influx is a characteristic feature. Indeed, HNE is believed to play a role in several pulmonary diseases, including chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), pulmonary emphysema, pneumonia, severe asthma, sarcoidosis, bronchiectasis and lung fibrosis. It is also implicated in several cardiovascular diseases in which tissue remodelling is involved, for example, in heart failure and the generation of ischaemic tissue injury following acute myocardial infarction. Elevated HNE levels are also correlated with the severity of inflammation in inflammatory bowel disease (Silberer H et al, Clin Lab. 2005;51(3-4):117-26) and may play a role in impaired mucosal repair in patients with ulcerative colitis.

COPD is an umbrella term encompassing three different pathological conditions, all of which contribute to limitation of airflow: chronic bronchitis, emphysema and small-airway disease. Generally all three will exist to varying extents in patients presenting with COPD, and all three may be due to neutrophil-mediated inflammation, as supported by the increased number of neutrophils observed in bronchoalveolar leakage (BAL) fluids of COPD patients (Thompson, A. B.; Daughton, D.; et al. *Am. Rev. Respir. Dis.* 1989, *140*, 1527-1537). The major pathogenic determinant in COPD has long been considered to be

the protease-anti-protease balance (also known as the 'elastase:anti-elastase hypothesis'), in which an imbalance of HNE and endogenous antiproteases such as α1-antitrypsin (α_1-AT), Secretory leukocyte protease inhibitor (SLPI) and pre-elafin leads to the various inflammatory disorders of COPD. Individuals that have a genetic deficiency of the protease inhibitor α1-antitrypsin (α1-AT) develop emphysema that increases in severity over time (Laurrell, C. B.; Erikkson, S *Scand. J. Clin. Invest.* 1963 *15*, 132-140). An excess of HNE is therefore destructive, leading to the breakdown of pulmonary morphology with loss of elasticity and destruction of alveolar attachments of airways in the lung (emphysema) whilst simultaneously increasing microvascular permeability and mucus hypersecretion (chronic bronchitis).

Multimeric ligands consist of multiple binding domains which are tethered together through a suitable scaffold. Hence individual binding domains are linked together into a single molecule, increasing the probability that the multimer will bind simultaneously with multiple active sites resulting in high-affinity interactions (Handl, H. L. *et al. Expert Opin. Ther. Targets* 2004, *8*, 565-586; Han, Y. F. *et al., Bioorg. Med. Chem. Letts.* 1999, *7*, 2569-2575). Also, multiple binding interactions with relatively high off-rates can combine to yield an overall low off-rate for the multimeric ligand. Thus, a molecule consisting of a suitable linker and ligands may be expected to show advantage over the monomeric ligands alone in terms of potency and/or duration of action. Multimeric compounds are unlikely to be orally bioavailable (as predicted by Lipinski's "Rule of 5") which may be advantageous where an inhaled route of administration to the lungs is targeted, since even after inhaled administration, a large proportion of drug is likely to enter the GI tract. Thus such compounds may be expected to show reduced systemic exposure after inhalation administration and hence an improved toxicity profile over orally administered therapies.

Monomers of formula (II) are described as inhibitors of human neutrophil elastase in WO2004/024700, WO2004/024701, GB2392910, WO2005/082863 and WO2005/082864.

Summary of the Invention

A first aspect of the invention is a compound of formula (I) or formula (IV):

$$(M)\text{-}(L)\text{-}(M) \qquad (I)$$

$$[(M)\text{-}(L)]_t\text{-}G \qquad (IV)$$

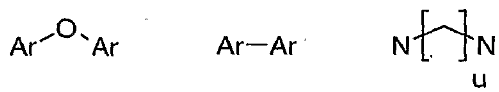
wherein

each M is independently an inhibitor of HNE; and

each L is independently a linker group;

t is 2 to 20; and

G is aryl, heteroaryl, alkyl, cycloalkyl, nitrogen, a dendrimer or a group of any of formulae (V) to (VII):



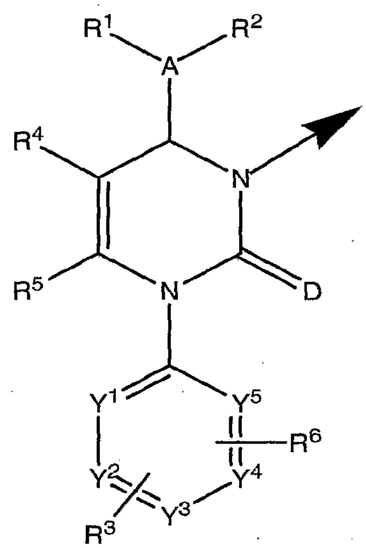
(V) (VI) (VII)

wherein

Ar is aryl or heteroaryl; and

u is 2-20;

M is a group of Formula (II)



(II)

wherein

A is aryl or heteroaryl;

D is oxygen or sulphur;

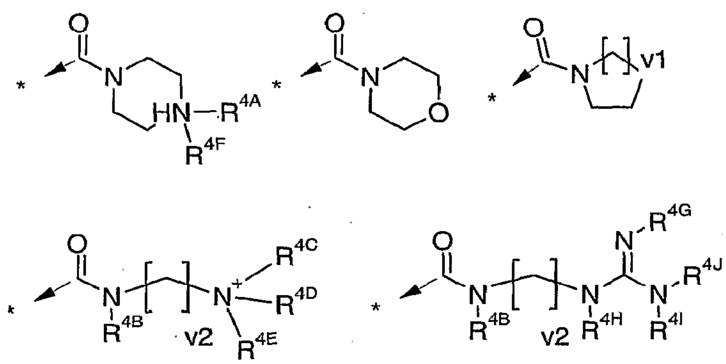
R^1, R^2 and R^3 are independently each hydrogen, halogen, nitro, cyano, alkyl, hydroxy or alkoxy, wherein alkyl and -alkoxy can be further substituted with one to three identical or different radicals selected from the group consisting of halogen, hydroxy and alkoxy;

R^4 is trifluoromethylcarbonyl, alkylcarbonyl, alkoxycarbonyl, alkenoxycarbonyl, hydroxycarbonyl, aminocarbonyl, arylcarbonyl, heteroarylcarbonyl, heterocycloalkylcarbonyl, heteroaryl, heterocycloalkyl or cyano, wherein

alkylcarbonyl, alkoxycarbonyl, and aminocarbonyl can be further substituted with one to three identical or different radicals selected from the group consisting of cycloalkyl, hydroxy, alkoxy, alkoxycarbonyl, hydroxycarbonyl, aminocarbonyl, cyano, amino, heteroaryl, heterocycloalkyl and tri-(alkyl)-silyl, and wherein heteroarylcarbonyl, heterocycloalkylcarbonyl, heteroaryl and heterocycloalkyl can be further substituted with alkyl;

or

R^4 represents a group of Formula (VIII);



.....(VIII)

Wherein

R^{4A}, R^{4B}, R^{4G}, R^{4H}, R^{4I} and R^{4J} are independently hydrogen or alkyl, or R^{4H} and R^{4I} may may be joined together with the nitrogen atom to which they are attached to form a ring;

R^{4F} is a lone pair or R^{4F} is alkyl and the nitrogen atom to which it is attached is quaternary and carries a positive charge;

R^{4C}, R^{4D} and R^{4E} are alkyl, or any two of R^{4C}, R^{4D} or R^{4E} may be joined together with the nitrogen atom to which they are attached to form a ring, optionally containing a further heteroatom selected from oxygen or nitrogen;

v1 is 1-3;

v2 is 1-6

R^5 is alkyl, which can be substituted with one to three identical or different radicals selected from the group consisting of halogen, hydroxy, alkoxy, alkenoxy, alkylthio, amino, hydroxycarbonyl, alkoxycarbonyl and the radical –O-(alkyl)-O-(alkyl); or R^5 is amino;

R^6 is halogen, nitro, cyano, alkyl, hydroxy or alkoxy, wherein alkyl and alkoxy can be further substituted with one to three identical or different radicals

selected from the group consisting of halogen, hydroxy and alkoxy; and

Y^1, Y^2, Y^3, Y^4 and Y^5 are independently each C or N, with the proviso that the ring in which they are comprised contains no more than 2 N atoms

L is a linker group of Formula (III),

$$- L^a - R^7 - L^b - W - L^b - R^7 - L^a - \ldots\text{(III)}$$

wherein;

L^a is a bond or group –C(O)-;

L^b is a bond or group –C(O)-;

R^7 is an alkylene or cycloalkylene group;

W is a bond or is selected from the following divalent radicals

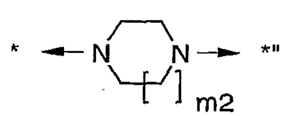
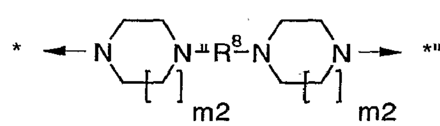
$-(O - R^{8A})_{m1} - O-$

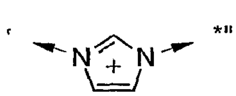
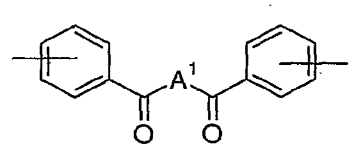
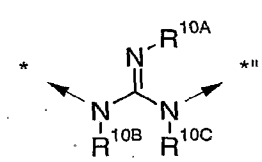
$-N(R^{9A}) - (O - R^{8A})_{m1} - R^{8A} - N(R^{9A})-$

$-N(R^{9A}) - R^{8B} - N(R^{9B})(R^{9C}) - R^{8B} - N(R^{9A})-$

$-N(R^{9A}) - R^{8B} - N(R^{10B})C(=NR^{10A})(NR^{10C}) - R^{8B} - N(R^{9A})-$

$-N(R^{9A}) - R^{8B} - N(R^{9A})-$

 ; 

 ;  ; 

Wherein;

m1 is 1-4;

R^{8A} is an alkylene or cycloalkylene group;

R^{8B} is an alkylene or cycloalkylene group, or a group of Formula A^2;

R^{9A} is hydrogen or alkyl;

One of R^{9B} or R^{9C} is a lone pair and the other is hydrogen or alkyl, or R^{9B} and R^{9C} are both alkyl, in which case the nitrogen to which they are attached is quaternary and carries a positive charge. Additionally, R^{9B} and R^{9C} may be joined together with the nitrogen to which they are attached to form a ring;

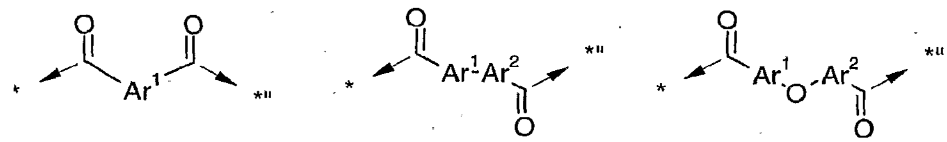
R^{10A} is hydrogen or alkyl;

R^{10B} and R^{10C} are independently hydrogen or alkyl, or alternatively R^{10B} and R^{10C} may be joined together to form a ring;

m2 is 1-3;

A^1 is selected from the groups $-N(R^{9A})-R^8-N(R^{9B})(R^{9C})-R^8-N(R^{9A})-$, $-N(R^{9A})-R^8-N(R^{10B})C(=NR^{10A})(NR^{10C})-R^8-N(R^{9A})-$;

A^2 is selected from the groups of Formula (...)



Wherein Ar^1, Ar^2 are independently an aryl or heteroaryl group.

or a pharmaceutically acceptable salt, solvate or N-oxide thereof.

Compounds of the invention may be described as dimers, when there are two groups M. The linker L may however carry one or more further groups M.

It will be appreciated that any compound of the invention may be used in the form of a prodrug.

Compounds of the invention may be useful in the treatment or prevention of diseases in which HNE is implicated, for example chronic obstructive pulmonary disease (COPD), chronic bronchitis, lung fibrosis, pneumonia, acute respiratory distress syndrome (ARDS), pulmonary emphysema, smoking-induced emphysema, severe asthma, sarcoidosis, bronchiectasis, cystic fibrosis, inflammatory bowel disease; ulcerative colitis and Crohn's disease.

Another aspect of the invention is a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier or excipient.

Another aspect of the invention is the use of a compound of the invention for the manufacture of a medicament for the treatment or prevention of a disease or condition in which HNE is implicated. Thus, compounds of the invention may be used in a method of therapy, for the treatment of a patient suffering from a condition or disease as defined above.

Description of Preferred Embodiments

"Alkylcarbonyl" means a –CO-alkyl group in which the alkyl group is as described herein. Exemplary acyl groups include $-COCH_3$ and $-COCH(CH_3)_2$.

"Acylamino" means a -NR-acyl group in which R and acyl are as described herein. Exemplary acylamino groups include $-NHCOCH_3$ and $-N(CH_3)COCH_3$.

"Alkenoxy" means an –O-alkenyl group in which alkenyl is as described below. Exemplary groups includes –O-allyl (-$OCH_2CH=CH_2$)

"Alkenoxycarbonyl" means a –COO-alkenyl group which alkenyl is as described below. Exemplary groups includes –C(O)O-allyl.

"Alkoxy" and "alkyloxy" means an -O-alkyl group in which alkyl is as described below. Exemplary alkoxy groups include methoxy (-OCH_3) and ethoxy (-OC_2H_5).

"Alkoxycarbonyl" means a -COO-alkyl group in which alkyl is as defined below. Exemplary alkoxycarbonyl groups include methoxycarbonyl and ethoxycarbonyl.

"Alkyl" or "lower alkyl", as a group or part of a group, refers to a straight or branched chain saturated hydrocarbon group having from 1 to 12, preferably 1 to 6, carbon atoms, in the chain. Exemplary alkyl groups include methyl, ethyl, 1-propyl and 2-propyl.

"Alkenyl" as a group or part of a group refers to a straight or branched chain hydrocarbon group having from 1 to 12, preferably 1 to 6, carbon atoms and one carbon-carbon double bond in the chain. Exemplary alkenyl groups include ethenyl, 1-propenyl, and 2-propenyl.

"Alkylamino" means a -NH-alkyl group in which alkyl is as defined above. Exemplary alkylamino groups include methylamino and ethylamino.

"Alkylene means an -alkyl- group in which alkyl is as defined previously. Exemplary alkylene groups include -CH_2-, -$(CH_2)_2$- and -$C(CH_3)HCH_2$-.

"Alkenylene" means an -alkenyl- group in which alkenyl is as defined previously. Exemplary alkenylene groups include –CH=CH-, -$CH=CHCH_2$-, and -$CH_2CH=CH$-.

"Alkylthio" means a -S-alkyl group in which alkyl is as defined above. Exemplary alkylthio groups include methylthio and ethylthio.

"Amino" means a –NR^1R^2 group where R^1 and R^2 may be independently a hydrogen atom, alkyl, aryl, arylalkyl, alkenyl, alkynyl, heteroaryl or heterocycloalkyl group. (i.e. The amino group may be primary, secondary or tertiary). Exemplary amino groups include –NH_2, $NHCH_3$, -NHPh, -$N(CH_3)_2$, etc.

"Aminocarbonyl" means a -CO-NRR group in which R is as herein described. Exemplary aminocarbonyl groups include -$CONH_2$, -$CONHCH_3$ and –CONH-phenyl.

"Aminoalkyl" means an alkyl-NH_2 group in which alkyl is as previously described. Exemplary aminoalkyl groups include –CH_2NH_2.

"Ammonium" means a quarternary nitrogen group –$N^+R^1R^2R^3$ where R^1, R^2 and R^3 are alkyl, aryl, alkenyl, arylalkyl, heteroaryl, heterocycloalkyl, and the nitrogen atom carries a formal positive charge.

"Aryl" as a group or part of a group denotes an optionally substituted monocyclic or multicyclic aromatic carbocyclic moiety of from 6 to 14 carbon atoms, preferably from 6

to 10 carbon atoms, such as phenyl or naphthyl. The aryl group may be substituted by one or more substituent groups.

"Arylalkyl" means an aryl-alkyl- group in which the aryl and alkyl moieties are as previously described. Exemplary arylalkyl groups include benzyl, phenethyl and naphthlenemethyl.

"Arylalkyloxy" means an aryl-alkyloxy- group in which the aryl and alkyloxy moieties are as previously described. Preferred arylalkyloxy groups contain a C_{1-4} alkyl moiety. Exemplary arylalkyl groups include benzyloxy.

"Arylcarbonyl" means an aromatic ring joined to a carbonyl group –(C=O). Exemplary groups include benzoyl (-C(O)Ph).

"Aryloxy" means an -O-aryl group in which aryl is described above. Exemplary aryloxy groups include phenoxy.

"Cyclic amine" means an optionally substituted 3 to 8 membered monocyclic cycloalkyl ring system where one of the ring carbon atoms is replaced by nitrogen, and which may optionally contain an additional heteroatom selected from O, S or NR (where R is as described herein). Exemplary cyclic amines include pyrrolidine, piperidine, morpholine, piperazine and *N*-methylpiperazine. The cyclic amine group may be substituted by one or more substituent groups.

"Cycloalkyl" means an optionally substituted saturated monocyclic or bicyclic ring system of from 3 to 12 carbon atoms, preferably from 3 to 8 carbon atoms, and more preferably from 3 to 6 carbon atoms. Exemplary monocyclic cycloalkyl rings include cyclopropyl, cyclopentyl, cyclohexyl and cycloheptyl. The cycloalkyl group may be substituted by one or more substituent groups.

"Cyloalkylene" means means an optionally substituted saturated monocyclic or bicyclic ring system of from 3 to 12 carbon atoms, preferably from 3 to 8 carbon atoms, and more preferably from 3 to 6 carbon atoms, as a bivalent radical. Exemplary cycloalkylene groups include cyclohexane-1,4-diyl.

"Cycloalkylalkyl" means a cycloalkyl-alkyl- group in which the cycloalkyl and alkyl moieties are as previously described. Exemplary monocyclic cycloalkylalkyl groups include cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl and cycloheptylmethyl. "Dendrimer" means a multifunctional core group with a branching group attached to each functional site. Each branching site can be attached to another branching molecule and this process may be repeated multiple times.

"Halo" or "halogen" means fluoro, chloro, bromo, or iodo.

"Haloalkoxy" means an -O-alkyl group in which the alkyl is substituted by one or more halogen atoms. Exemplary haloalkyl groups include trifluoromethoxy and

difluoromethoxy.

"Haloalkyl" means an alkyl group which is substituted by one or more halo atoms. Exemplary haloalkyl groups include trifluoromethyl.

"Heteroaryl" as a group or part of a group denotes an optionally substituted aromatic monocyclic or multicyclic organic moiety of from 5 to 14 ring atoms, preferably from 5 to 10 ring atoms, in which one or more of the ring atoms is/are element(s) other than carbon, for example nitrogen, oxygen or sulfur. Examples of such groups include benzimidazolyl, benzoxazolyl, benzothiazolyl, benzofuranyl, benzothienyl, furyl, imidazolyl, indolyl, indolizinyl, isoxazolyl, isoquinolinyl, isothiazolyl, oxazolyl, oxadiazolyl, pyrazinyl, pyridazinyl, pyrazolyl, pyridyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, tetrazolyl, 1,3,4-thiadiazolyl, thiazolyl, thienyl and triazolyl groups. The heteroaryl group may be substituted by one or more substituent groups. The heteroaryl group may be attached to the remainder of the compound of the invention by any available carbon or nitrogen atom.

"Heteroarylcarbonyl" means a heteroaryl group attached to a carbonyl group –C(O)-. Exemplary groups are pyridine-2-carbonyl, thiophene-2-carbonyl.

"Heteroaryloxy" means a heteroaryloxy- group in which the heteroaryl is as previously described. Exemplary heteroaryloxy groups include pyridyloxy.

"Heterocycloalkyl" means: (i) an optionally substituted cycloalkyl group of from 4 to 8 ring members which contains one or more heteroatoms selected from O, S or NR; (ii) a cycloalkyl group of from 4 to 8 ring members which contains CONR and CONRCO (examples of such groups include succinimidyl and 2-oxopyrrolidinyl). The heterocycloalkyl group may be substituted by one or more substituent groups. The heterocycloalkyl group may be attached to the remainder of the compound by any available carbon or nitrogen atom.

"Heterocycloalkylalkyl" means a heterocycloalkyl-alkyl- group in which the heterocycloalkyl and alkyl moieties are as previously described.

"Hydroxycarbonyl" means a group –COOH.

"Pharmaceutically acceptable salt" means a physiologically or toxicologically tolerable salt and include, when appropriate, pharmaceutically acceptable base addition salts and pharmaceutically acceptable acid addition salts. For example (i) where a compound of the invention contains one or more acidic groups, for example carboxy groups, pharmaceutically acceptable base addition salts that may be formed include sodium, potassium, calcium, magnesium and ammonium salts, or salts with organic amines, such as, diethylamine, *N*-methyl-glucamine, diethanolamine or amino acids (e.g. lysine) and the like; (ii) where a compound of the invention contains a basic group, such as an amino group, pharmaceutically acceptable acid addition salts that may be formed

include hydrochlorides, hydrobromides, phosphates, acetates, citrates, lactates, tartrates, malonates, methanesulphonates and the like. "Pharmaceutically acceptable salt" also means quaternary ammonium salts. In this case, the acceptable salts may be chlorides, bromides, iodides, mesylates, tosylates, succinates and the like.

It will be understood that, as used herein, references to the compounds of the invention are meant to also include the pharmaceutically acceptable salts.

"Prodrug" refers to a compound which is convertible *in vivo* by metabolic means (e.g. by hydrolysis, reduction or oxidation) to a compound of the invention. For example an ester prodrug of a compound of the invention containing a hydroxy group may be convertible by hydrolysis *in vivo* to the parent molecule. Suitable esters of compounds of the invention containing a hydroxy group, are for example acetates, citrates, lactates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-bis-β-hydroxynaphthoates, gentisates, isethionates, di-*p*-toluoyltartrates, methanesulfonates, ethanesulfonates, benzenesulfonates, p-toluenesulfonates, naphthalene bis sulfonates, cyclohexylsulfamates and quinates. As another example an ester prodrug of a compound of the invention containing a carboxy group may be convertible by hydrolysis *in vivo* to the parent molecule. Examples of ester prodrugs are those described by F. J. Leinweber, Drug Metab. Res., 1987, 18, 379.

It will be understood that, as used in herein, references to the compounds of the invention are meant to also include the prodrug forms.

"Saturated" pertains to compounds and/or groups which do not have any carbon-carbon double bonds or carbon-carbon triple bonds.

The cyclic groups referred to above, namely, aryl, heteroaryl, cycloalkyl, and heterocycloalkyl, may be substituted by one or more substituent groups. Suitable optional substituent groups include acyl (e.g. $-COCH_3$), alkoxy (e.g., $-OCH_3$), alkoxycarbonyl (e.g. $-COOCH_3$), alkylamino (e.g. $-NHCH_3$), alkylsulfinyl (e.g. $-SOCH_3$), alkylsulfonyl (e.g. $-SO_2CH_3$), alkylthio (e.g. $-SCH_3$), $-NH_2$, aminoacyl (e.g. $-CON(CH_3)_2$), aminoalkyl (e.g. $-CH_2NH_2$), arylalkyl (e.g. $-CH_2Ph$ or $-CH_2-CH_2-Ph$), cyano, dialkylamino (e.g. $-N(CH_3)_2$), halo, haloalkoxy (e.g. $-OCF_3$ or $-OCHF_2$), haloalkyl (e.g. $-CF_3$), alkyl (e.g. $-CH_3$ or $-CH_2CH_3$), -OH, -CHO, $-NO_2$, aryl (optionally substituted with alkoxy, haloalkoxy, halogen, alkyl or haloalkyl), heteroaryl (optionally substituted with alkoxy, haloalkoxy, halogen, alkyl or haloalkyl), heterocycloalkyl, aminoacyl (e.g. $-CONH_2$, $-CONHCH_3$), aminosulfonyl (e.g. $-SO_2NH_2$, $-SO_2NHCH_3$), acylamino (e.g. $-NHCOCH_3$), sulfonylamino (e.g. $-NHSO_2CH_3$), heteroarylalkyl, cyclic amine (e.g. morpholine), aryloxy, heteroaryloxy, arylalkyloxy (e.g. benzyloxy) and heteroarylalkyloxy.

Alkylene or alkenylene groups may be optionally substituted. Suitable optional

substituent groups include alkoxy (e.g., $-OCH_3$), alkylamino (e.g. $-NHCH_3$), alkylsulfinyl (e.g. $-SOCH_3$), alkylsulfonyl (e.g. $-SO_2CH_3$), alkylthio (e.g. $-SCH_3$), $-NH_2$, aminoalkyl (e.g. $-CH_2NH_2$), arylalkyl (e.g. $-CH_2Ph$ or $-CH_2-CH_2-Ph$), cyano, dialkylamino (e.g. $-N(CH_3)_2$), halo, haloalkoxy (e.g. $-OCF_3$ or $-OCHF_2$), haloalkyl (e.g. $-CF_3$), alkyl (e.g. $-CH_3$ or $-CH_2CH_3$), -OH, -CHO, and $-NO_2$.

Compounds of the invention may exist in one or more geometrical, optical, enantiomeric, diastereomeric and tautomeric forms, including but not limited to *cis*- and *trans*-forms, *E*- and *Z*-forms, *R*-, *S*- and *meso*-forms, keto-, and enol-forms. Unless otherwise stated a reference to a particular compound includes all such isomeric forms, including racemic and other mixtures thereof. Where appropriate such isomers can be separated from their mixtures by the application or adaptation of known methods (e.g. chromatographic techniques and recrystallisation techniques). Where appropriate such isomers may be prepared by the application of adaptation of known methods (e.g. asymmetric synthesis).

Certain compounds and combinations of substituents are preferred. Certain preferences are given in the subclaims.

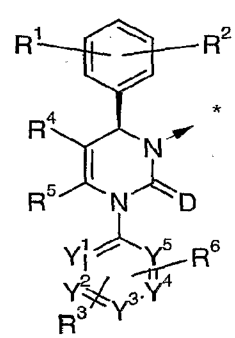
In a preferred embodiment, each M is the same or different and is a group of formula (II) as defined herein. In formula (II), the arrow denotes the point of attachment of M to the linker L. Preferably, L is a group of Formula (III) as defined herein.

In a preferred embodiment, compounds are of Formula (I).

In a preferred embodiment, A is a phenyl ring.

In a preferred embodiment D is an oxygen atom

In another preferred embodiment, the groups M have the stereochemistry shown below (R) configuration;



In one preferred embodiment R^6 is a haloalkyl group.

In one preferred embodiment, Y^1-Y^5 are carbon atoms.

In a preferred embodiment, L^a is a bond.

In a further preferred embodiment, W is the radical $-N(R^{9A}) - R^{8B} - N(R^{9B})(R^{9C}) - R^{8B} - N(R^{9A})-$.

In another preferred embodiment, W is the radical $-N(R^{9A}) - R^{8B} - N(R^{10B})C(=NR^{10A})(NR^{10C}) - R^{8B} - N(R^{9A})-$.

In yet another preferred embodiment R^5 is an alkyl group.

In yet another preferred embodiment R^5 is a methyl group.

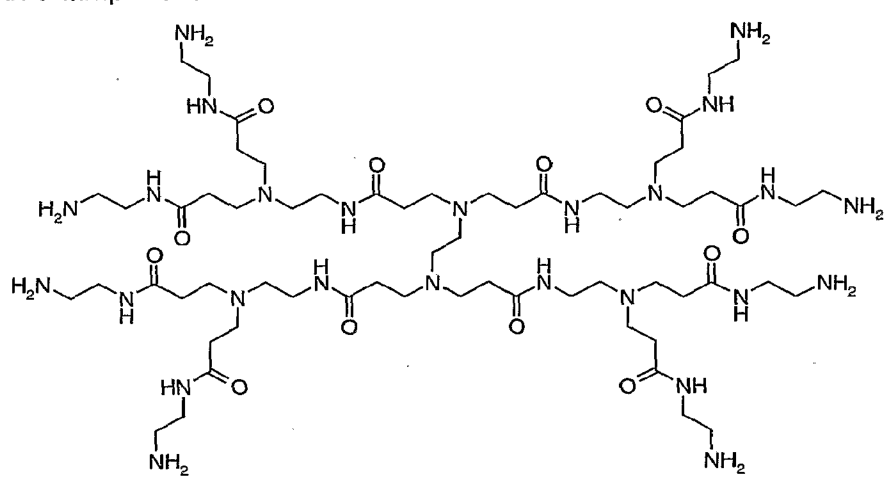
In one embodiment R^4 is alkoxycarbonyl

In a further embodiment R^4 is alkoxycarbonyl wherein the alkoxy group is substituted with a hydroxyl group.

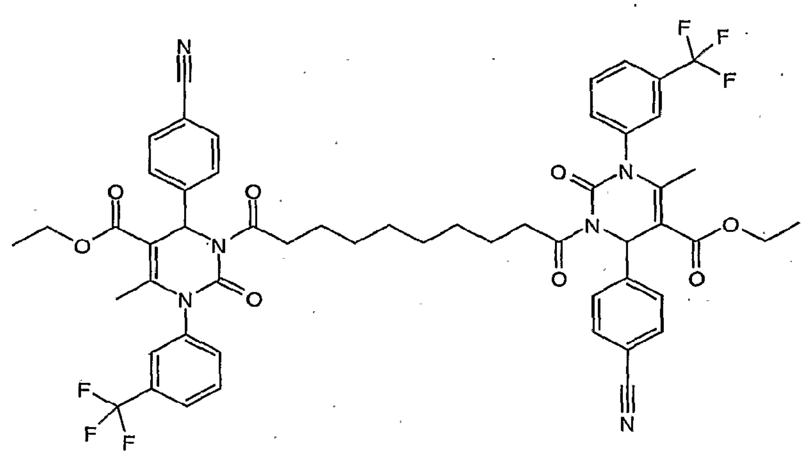
In a further embodiment R^4 is alkoxycarbonyl wherein the alkoxy group is substituted with an amino group.

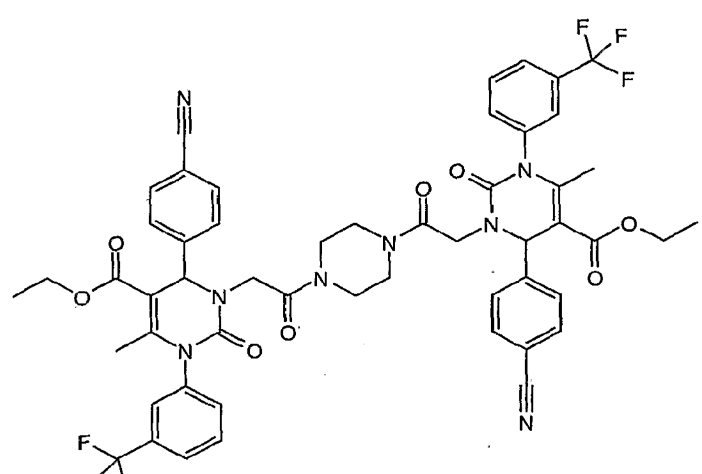
In a further embodiment R^4 is alkoxycarbonyl wherein the alkoxy group is substituted with an ammonium group.

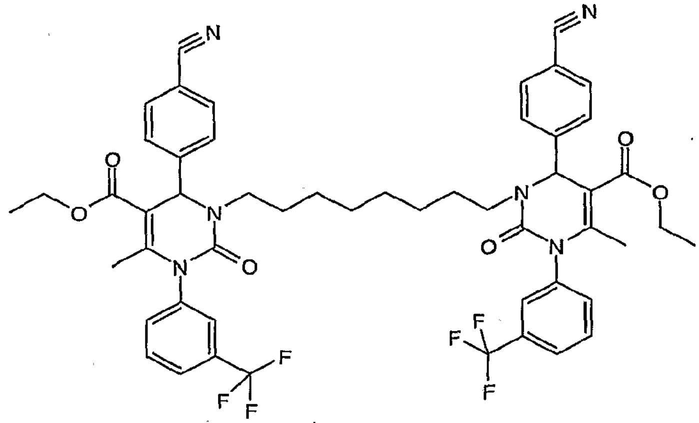
G may be a group of any of formulae (V) to (VII) or a dendrimer. Examples of groups of formulae (V) to (VII) include, but are not limited to phenoxyphenyl, biphenyl, bipyridyl, ethylenediamino, propylenediamino and the like. It is to be understood that the number of possible attachment points is dictated by the valency of the groups present, so that for example, biphenyl can contain up to 10 possible attachments (5 on each phenyl ring), and ethylenediamine can possess up to 4 possible attachments (2 on each terminal amine). An example of a dendrimer suitable for use in the invention is:

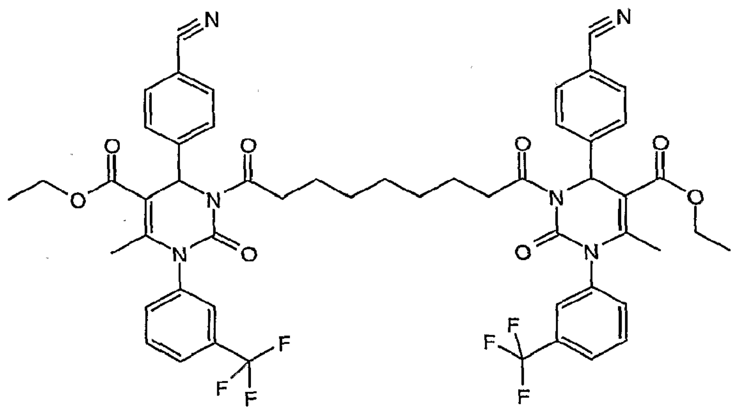


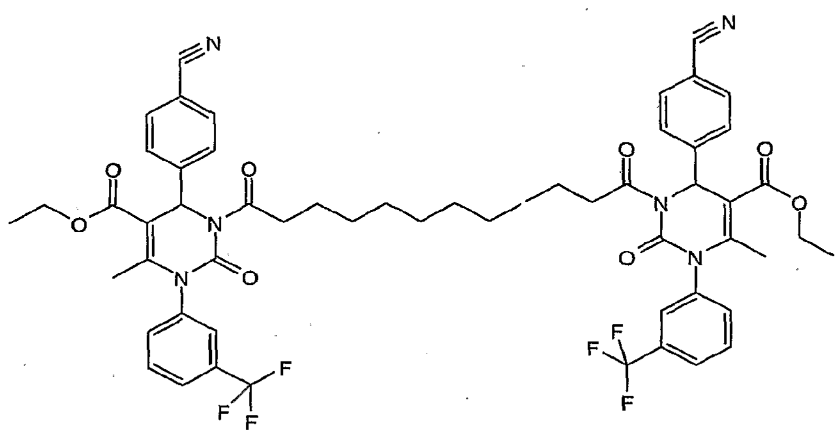
Preferred compounds of the invention include:

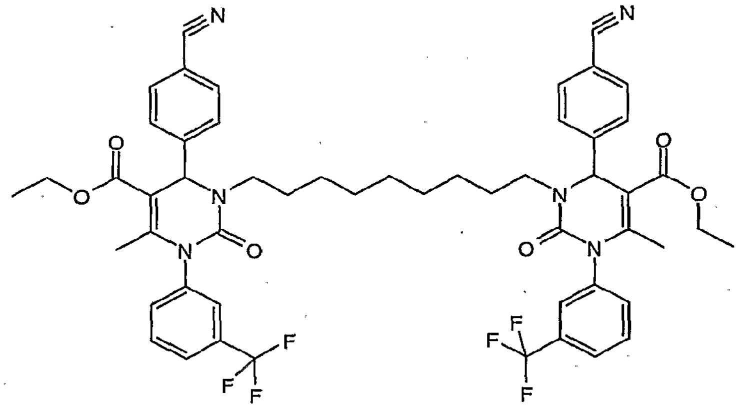


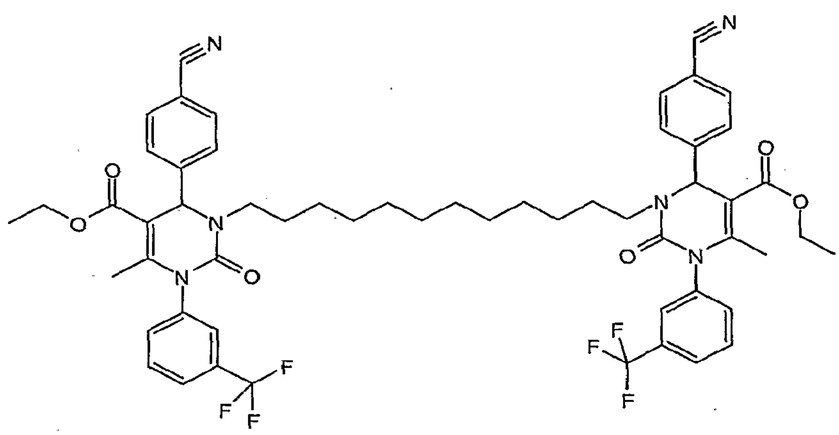


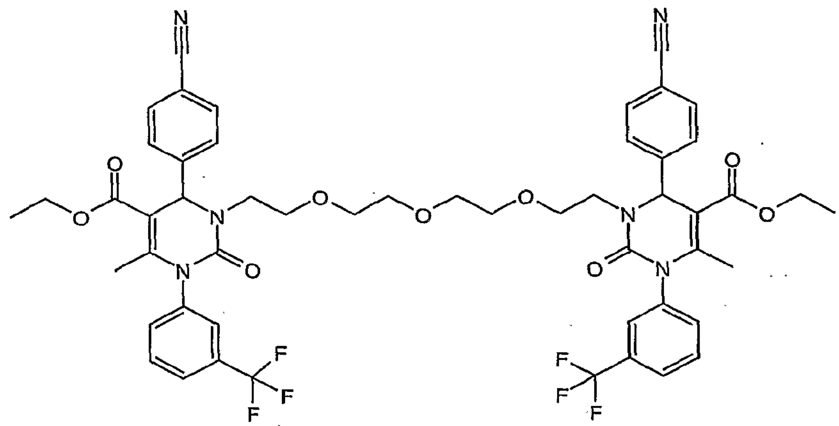


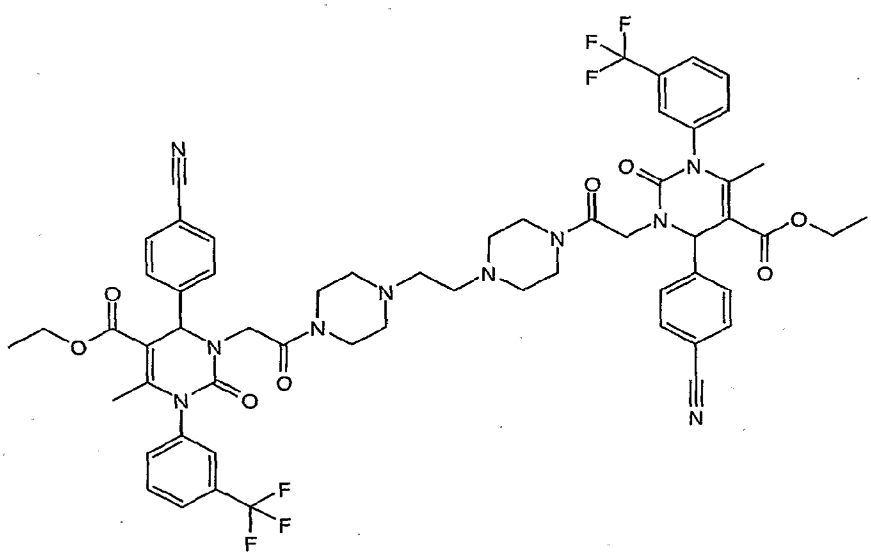


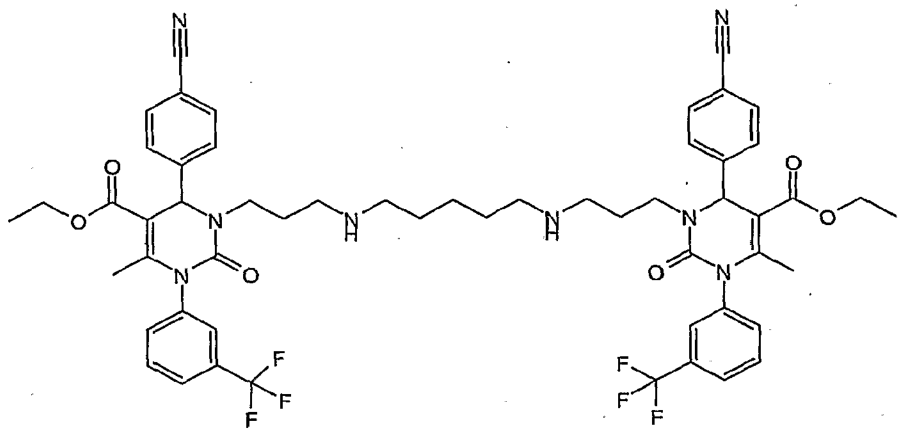


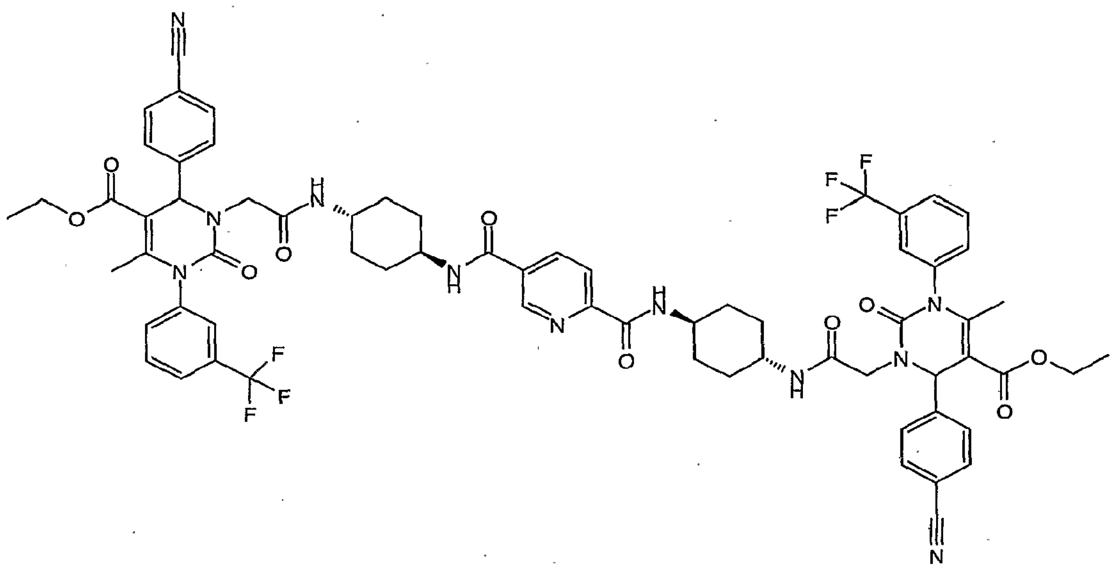


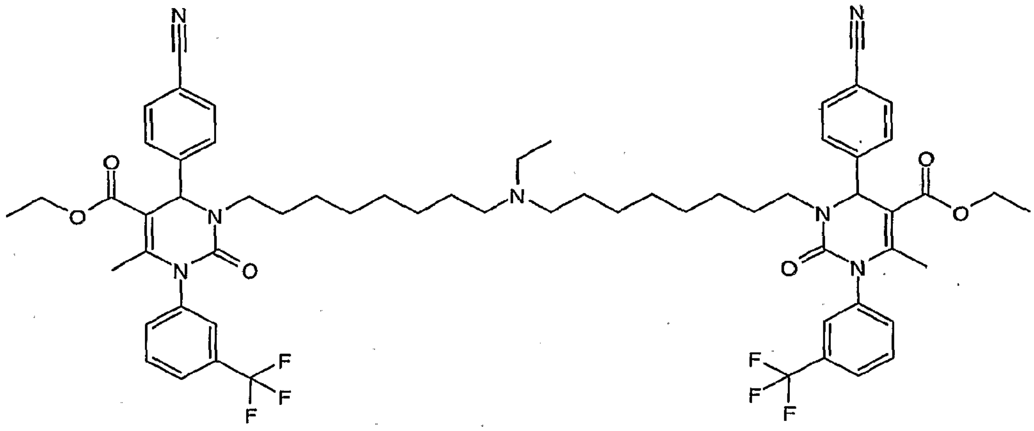


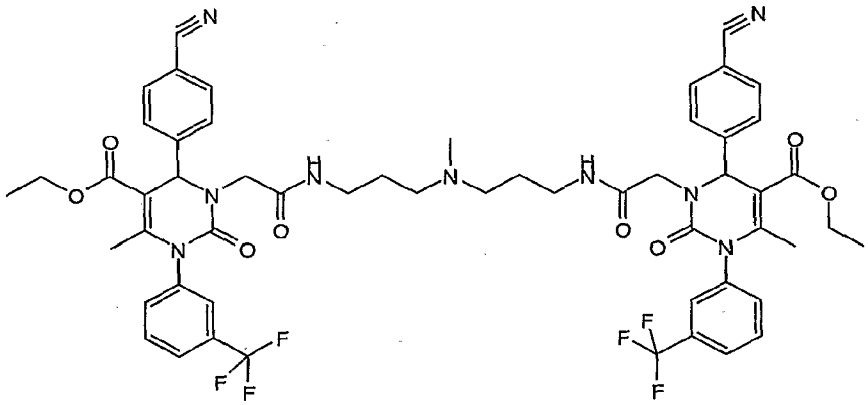


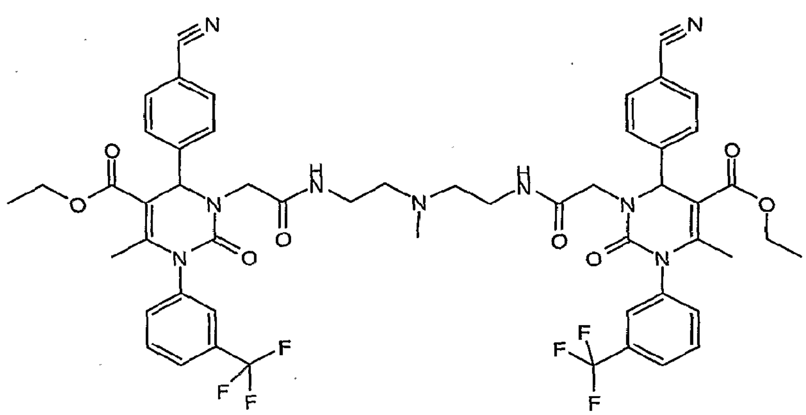


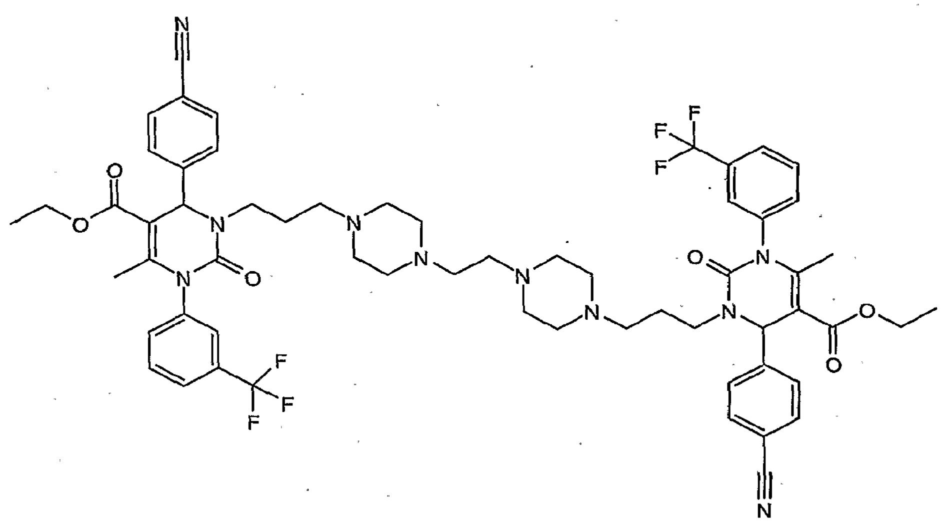


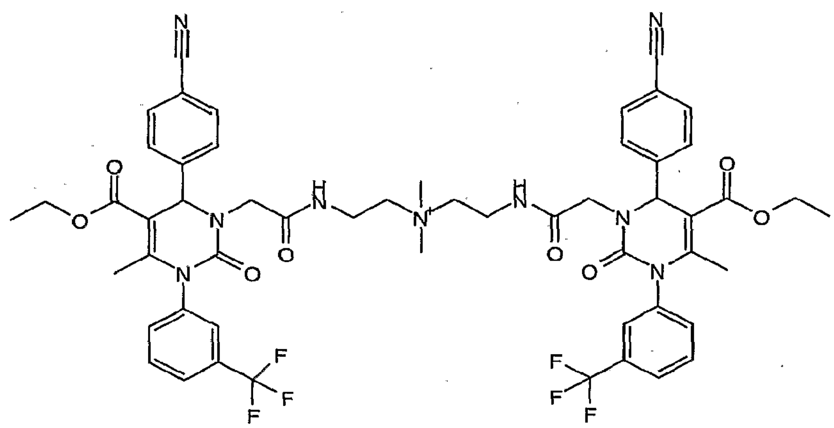


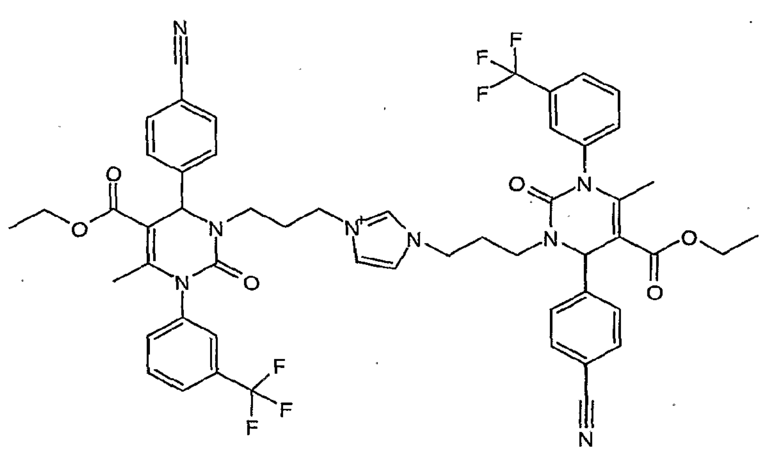


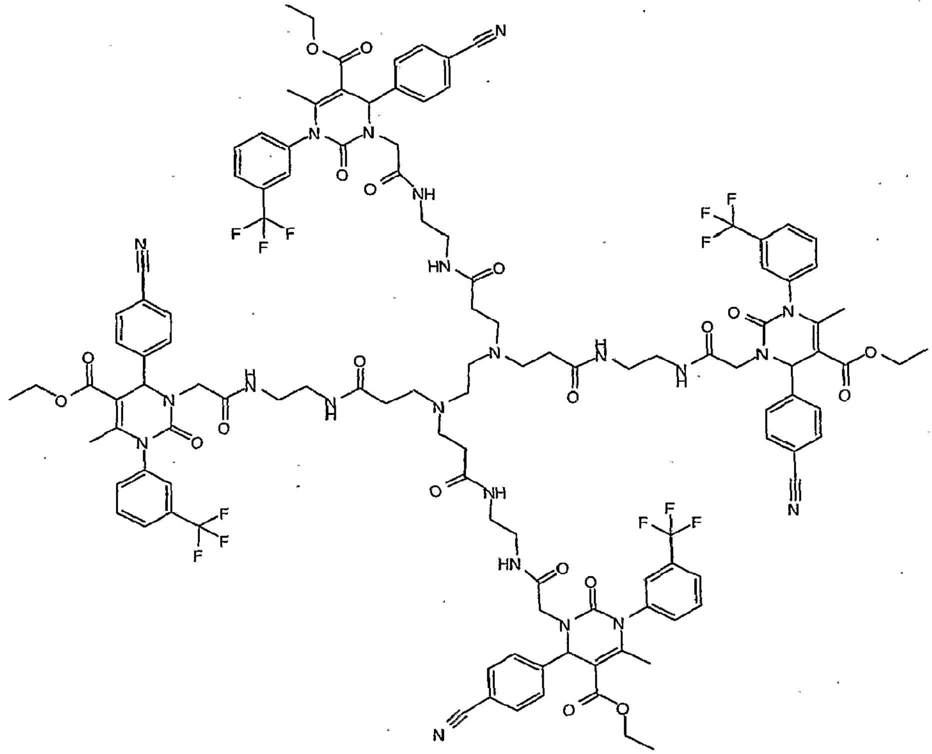


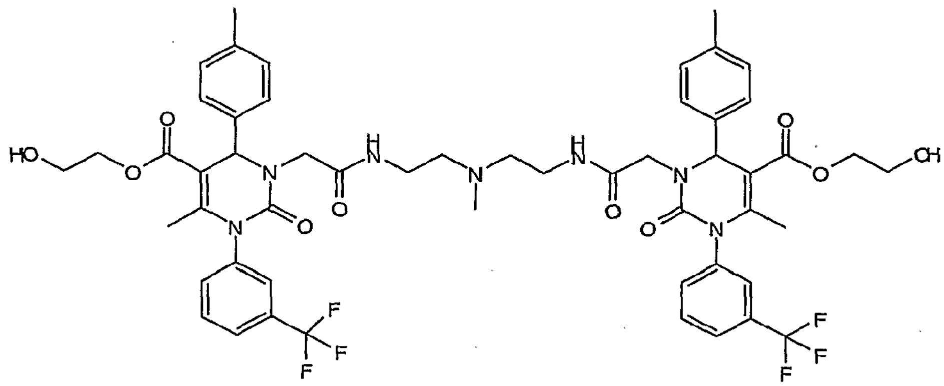


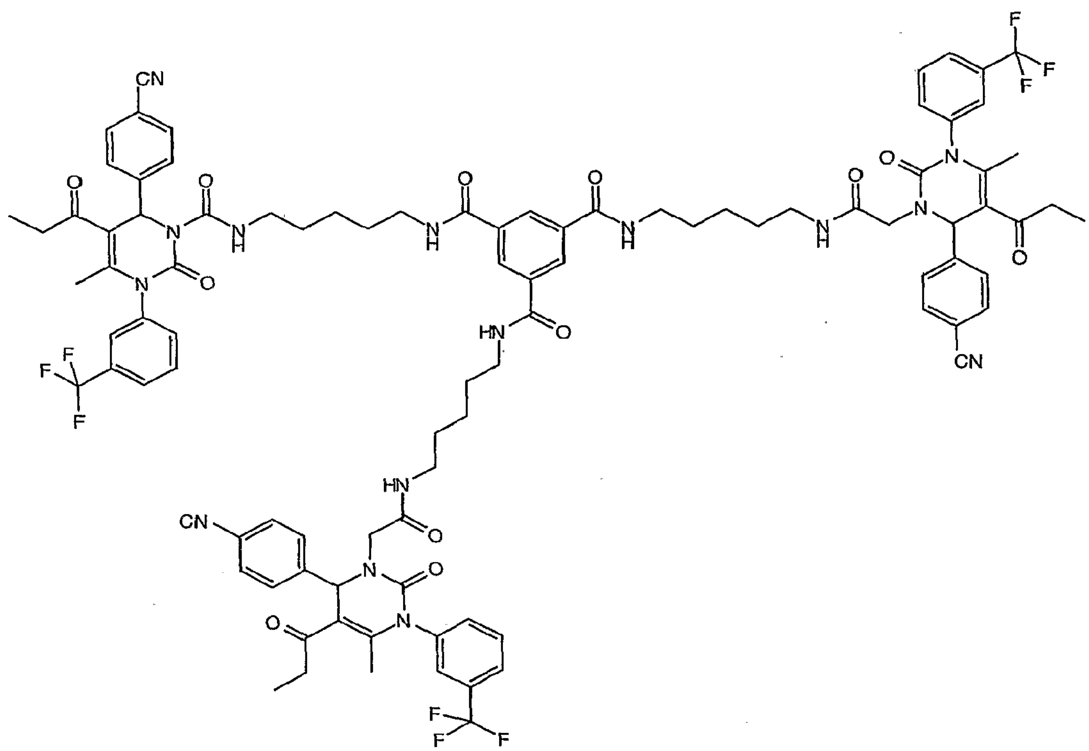


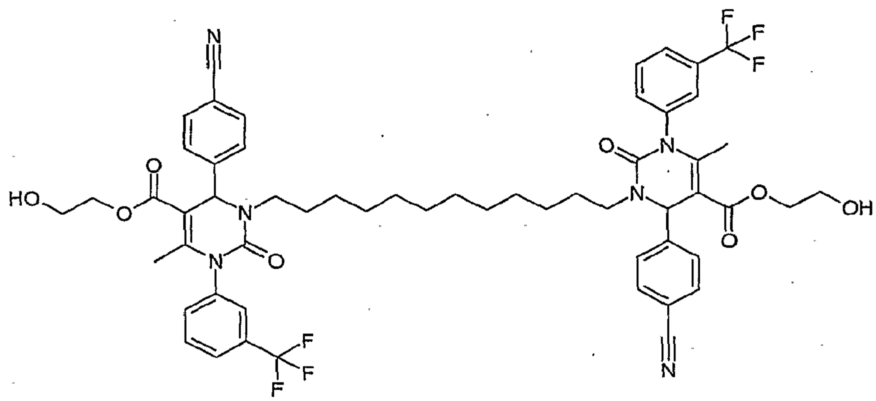


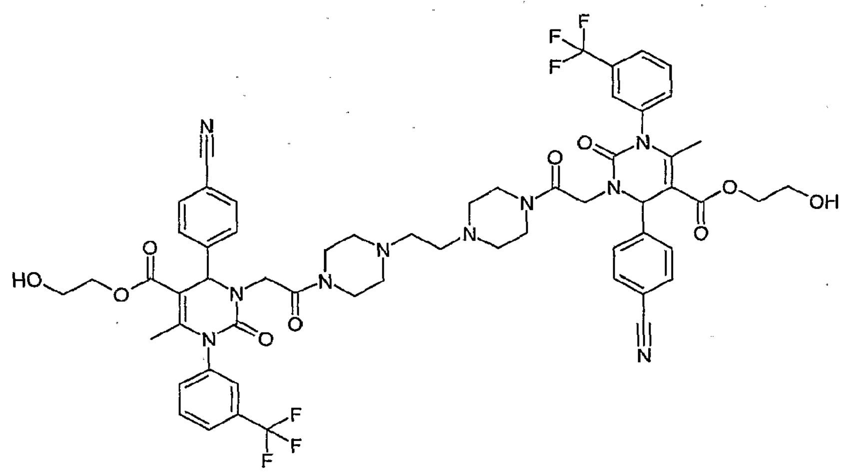


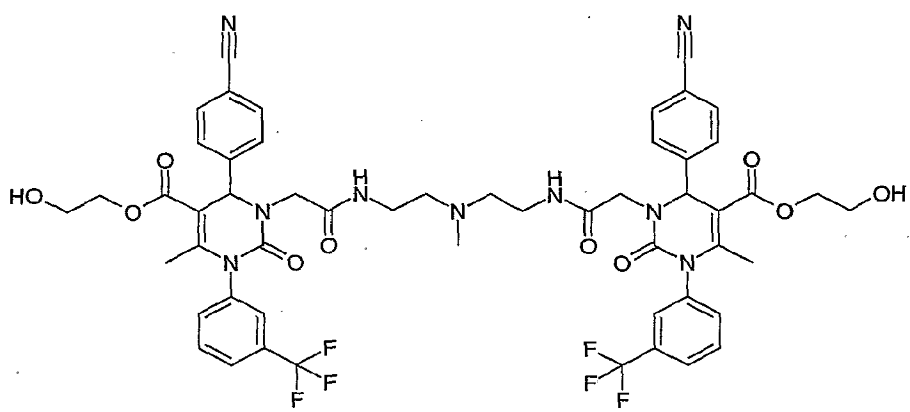


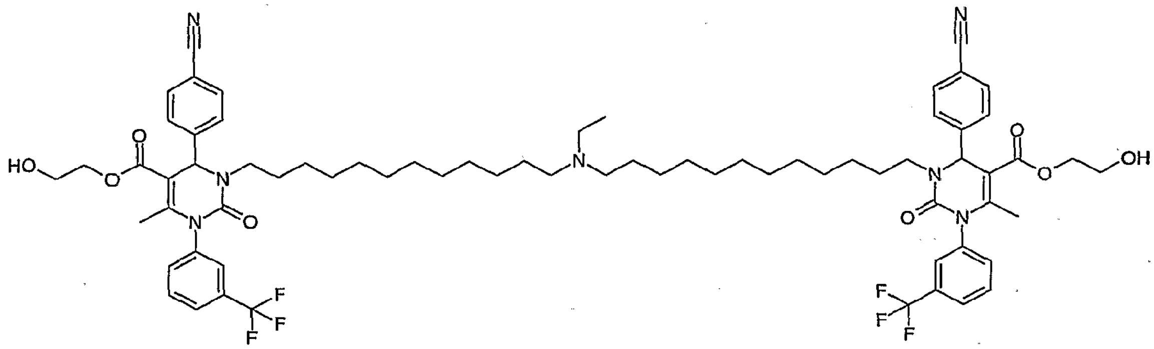


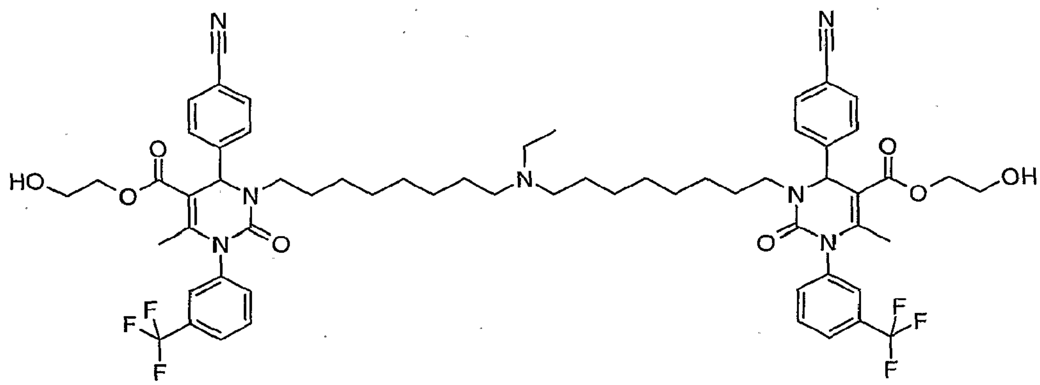


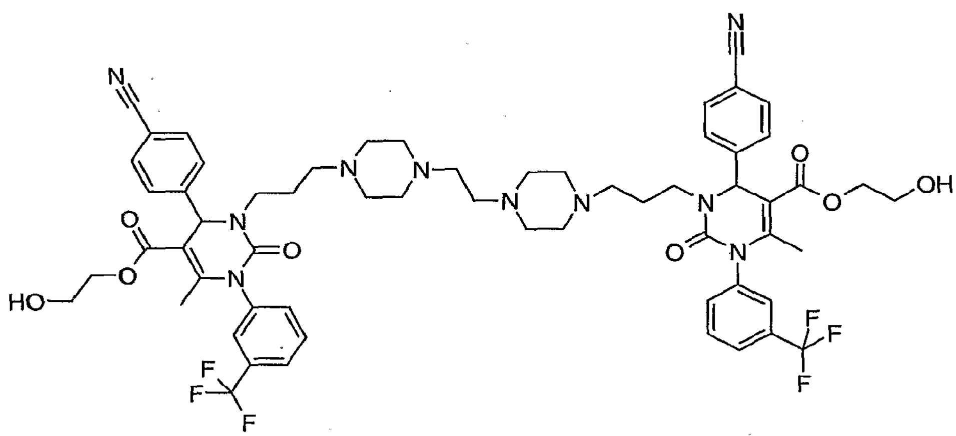


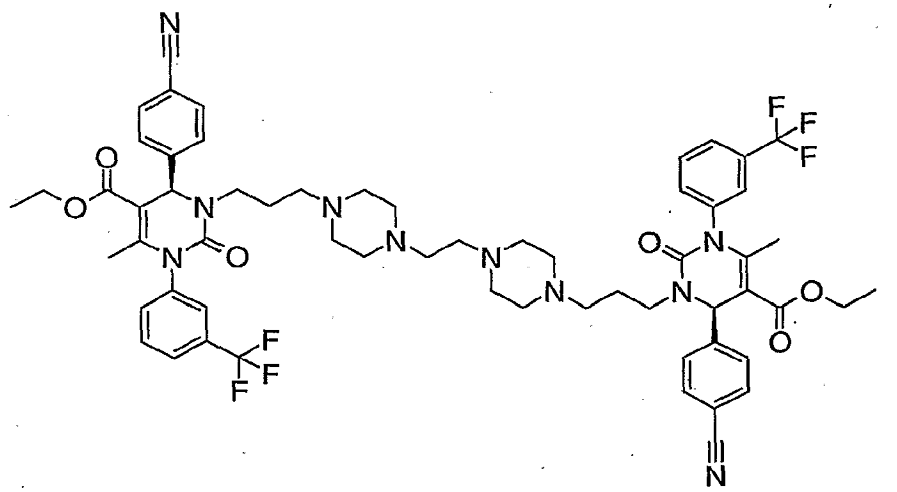


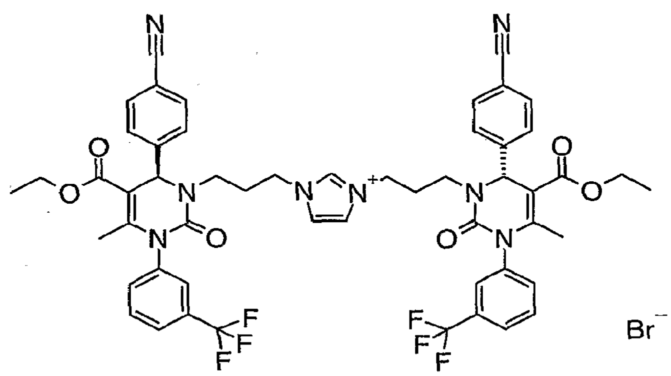


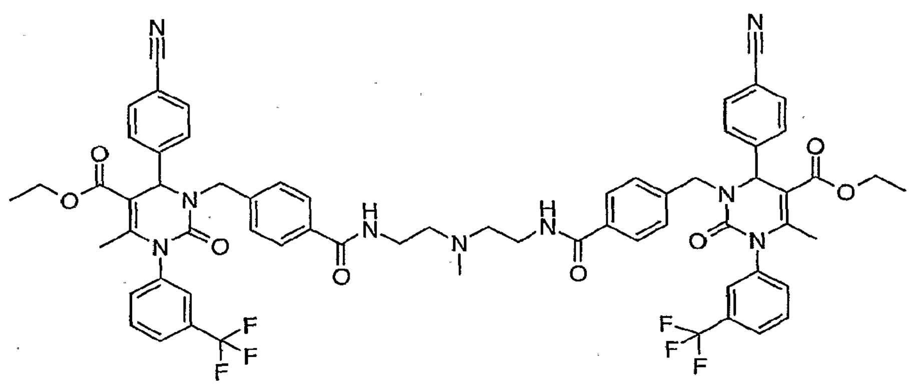


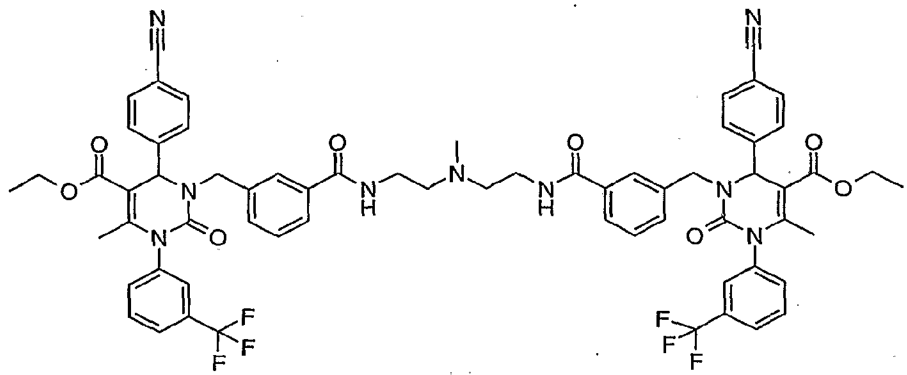


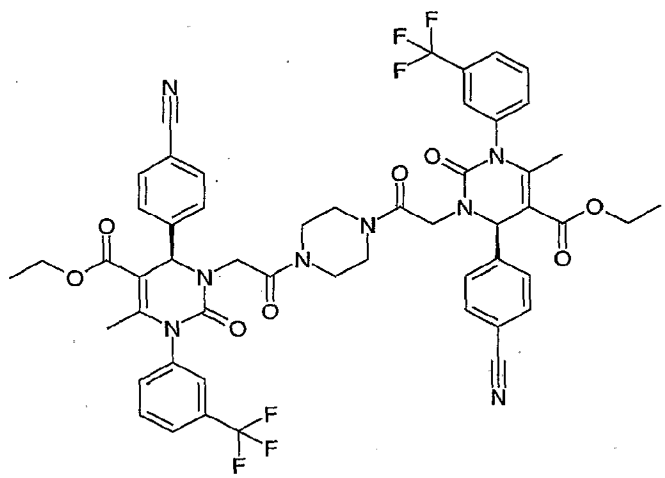


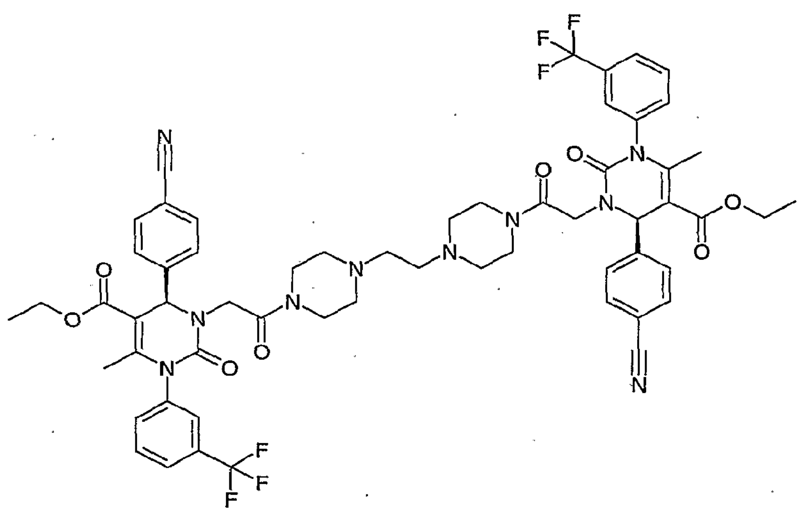


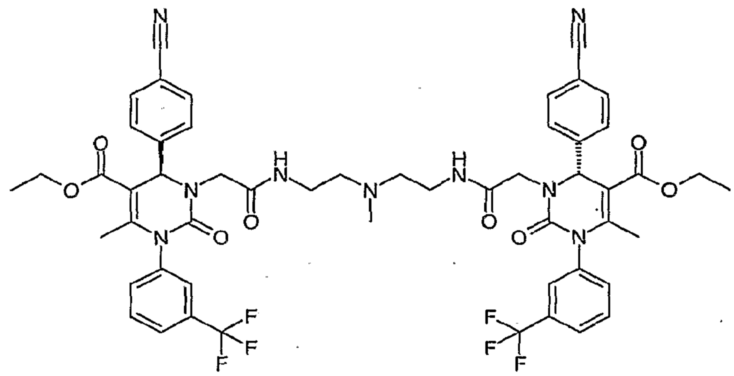


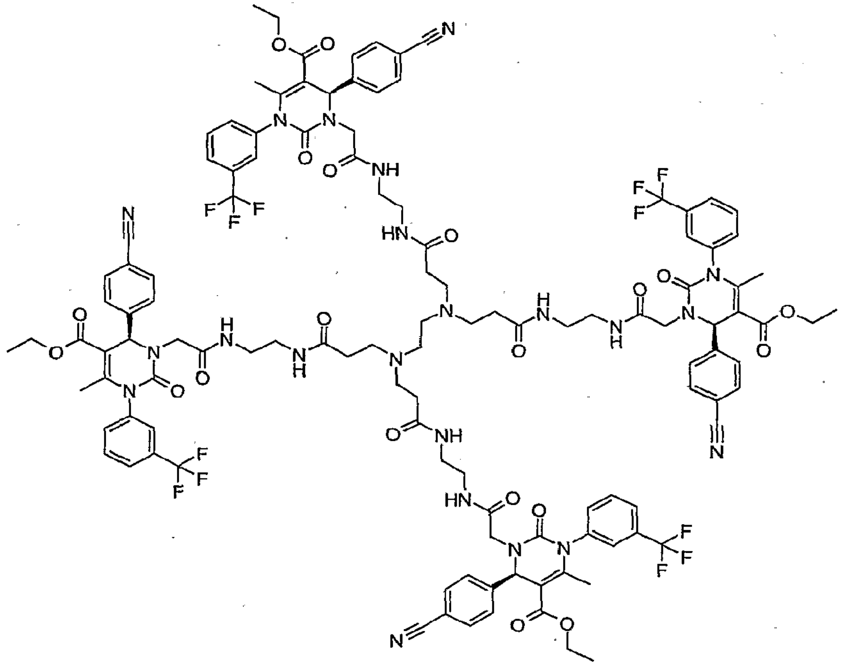


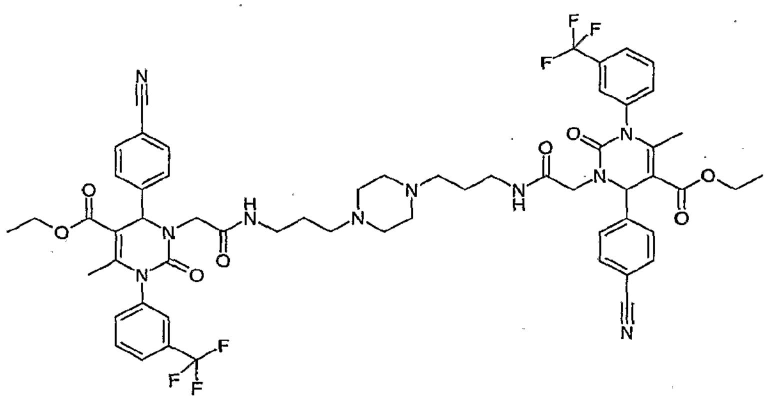


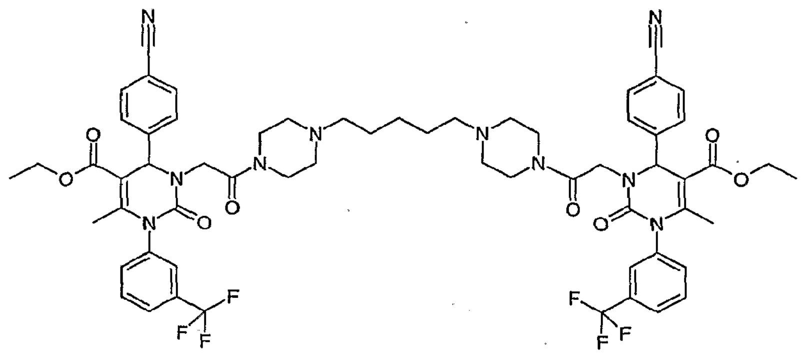


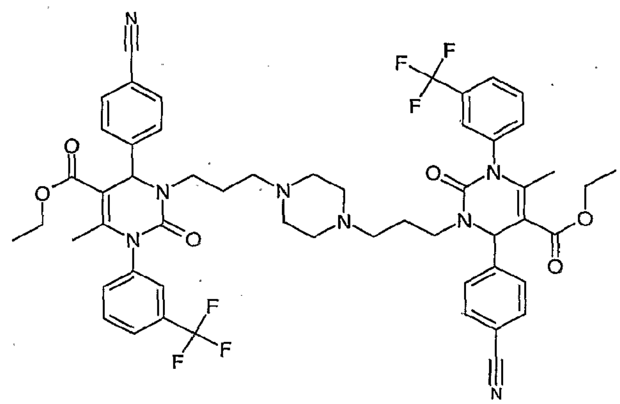


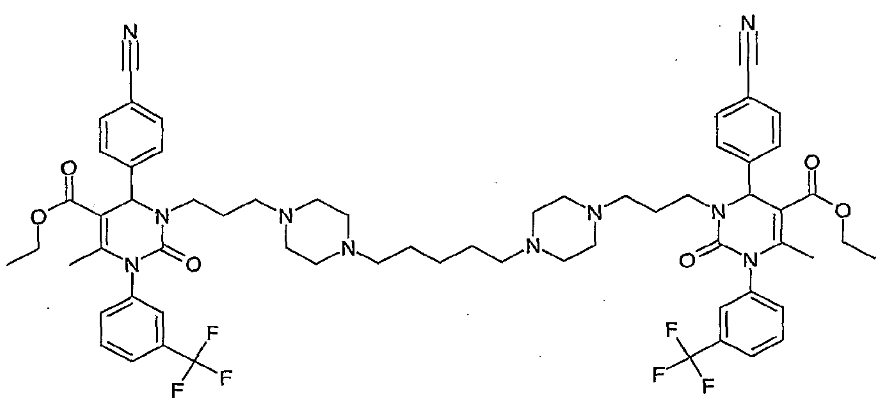


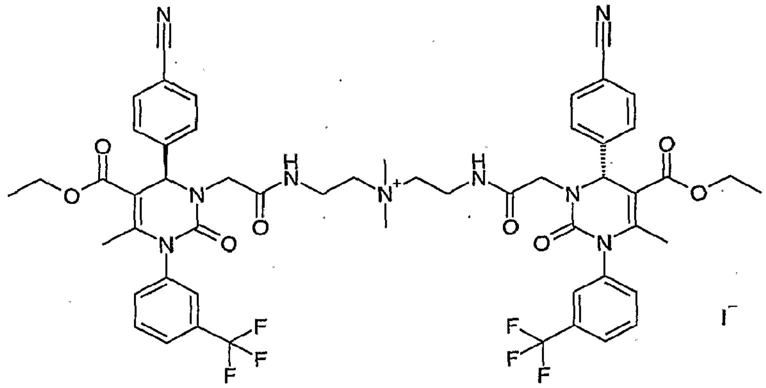


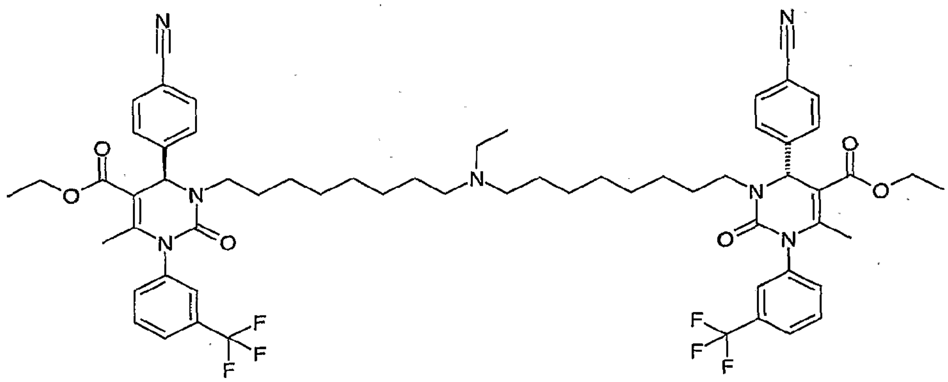


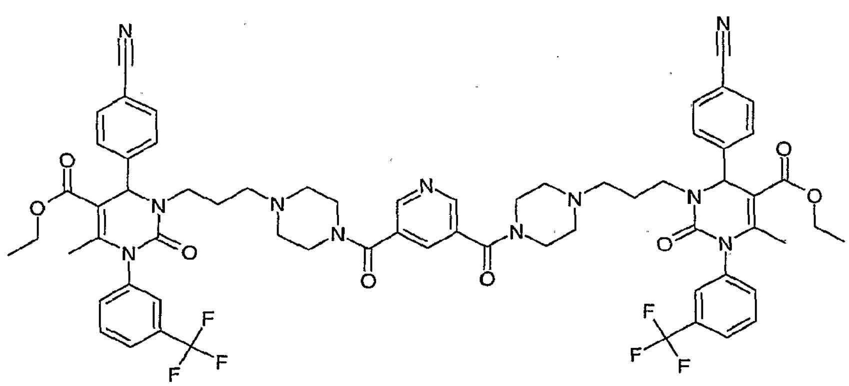


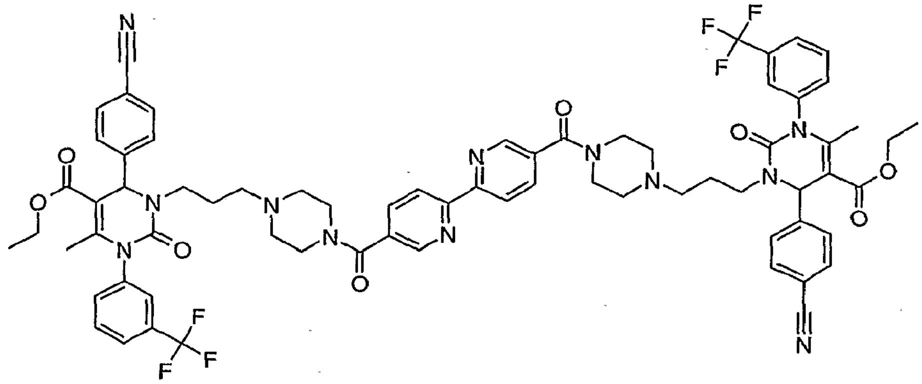


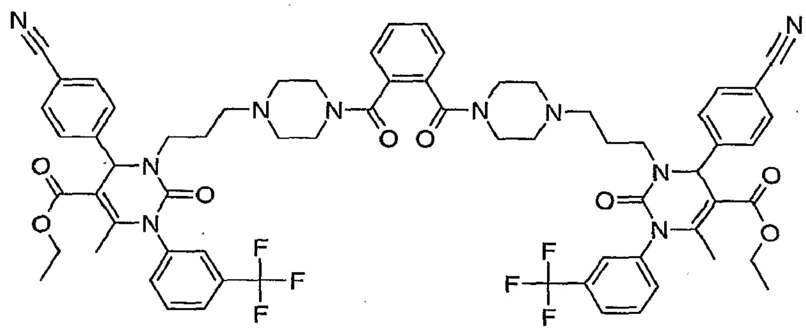


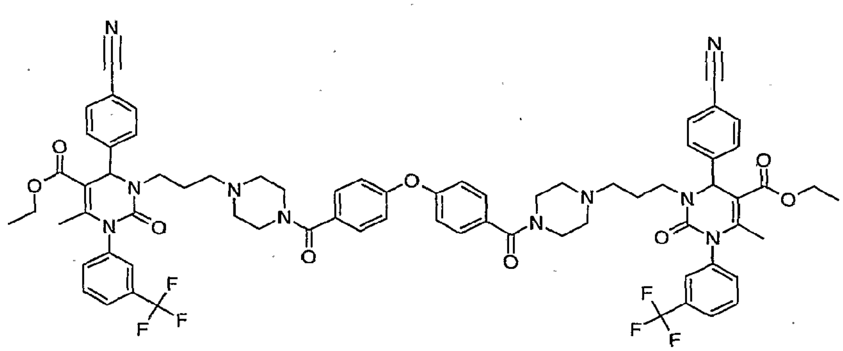


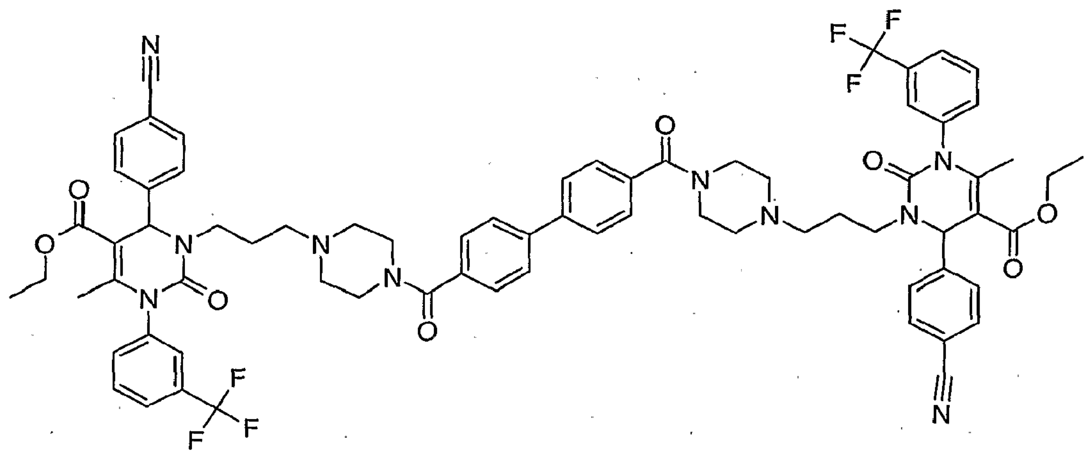


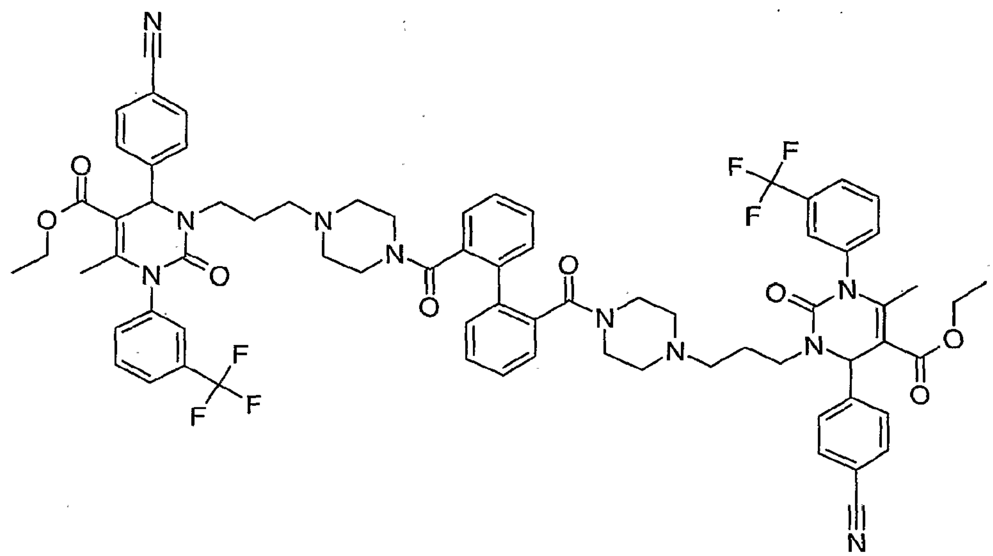


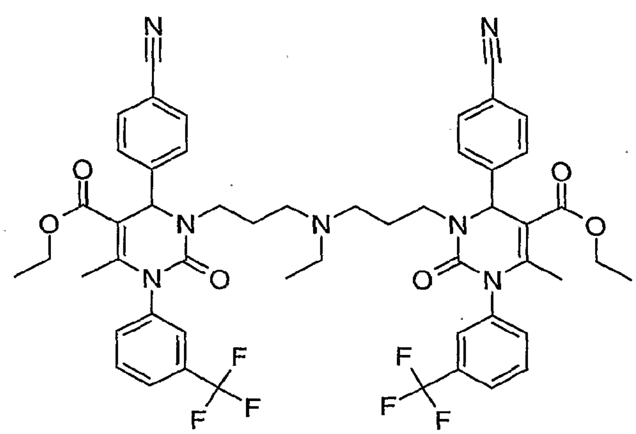


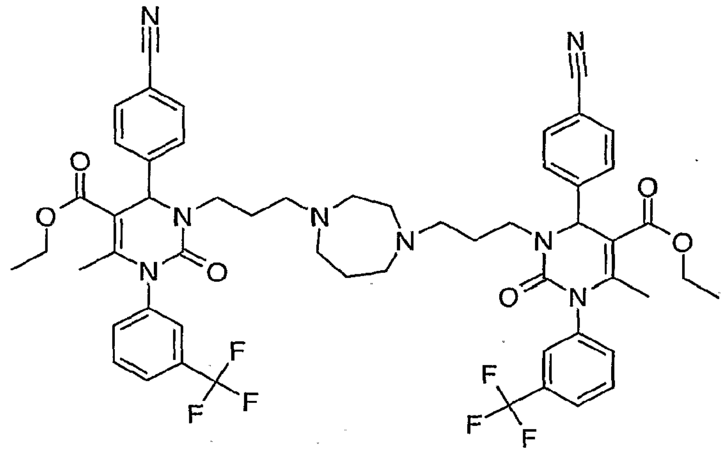


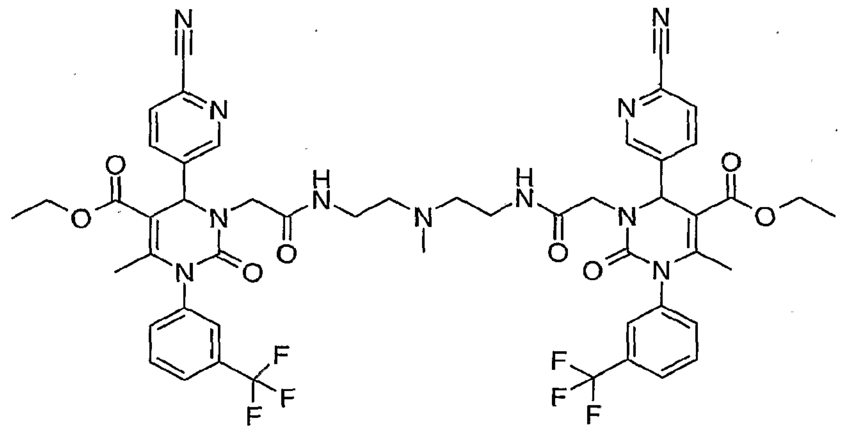


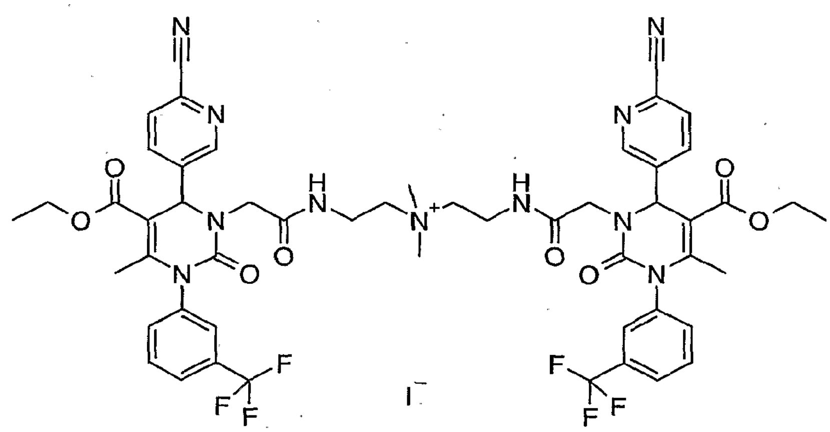

N
O
O
O
N
N
O
N
N
F
F
F
O
O
N
N
O
N
N
O
F
F
F
O
O
N

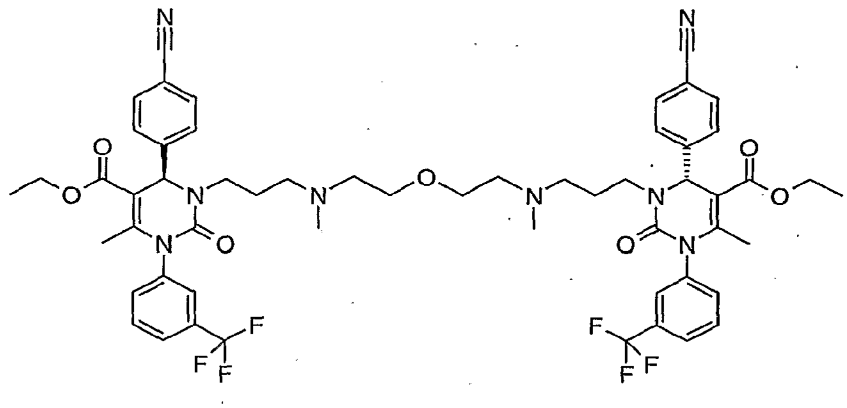

N
O
O
O
N
N
O
N
N
F
F
F
O
O
N
N
O
N
N
O
F
F
F
O
O
N

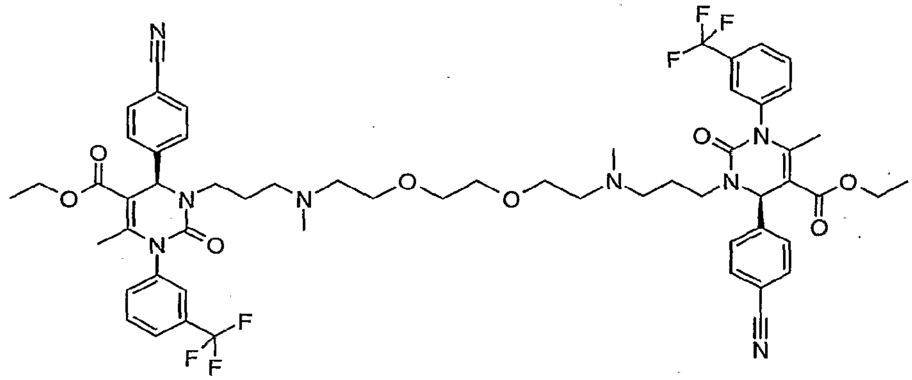

N
N
O
O
O
N
N
O
N
N
F
F
F
N
O
N
O
O
F
F
F

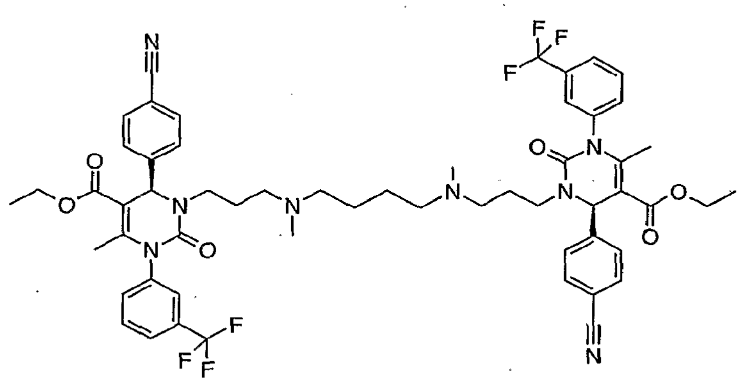


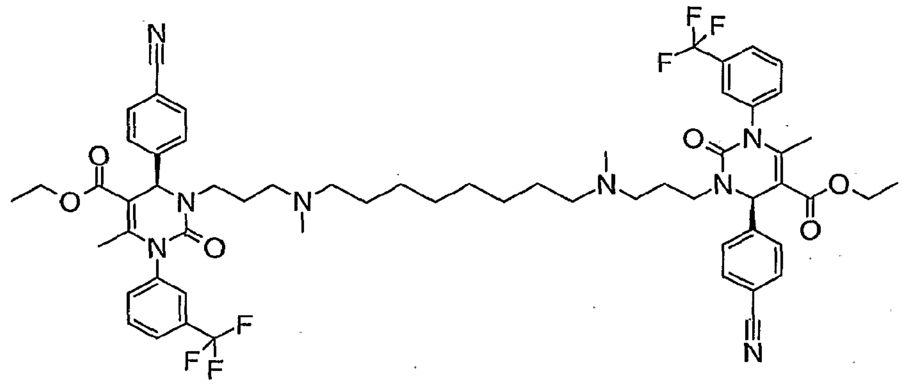


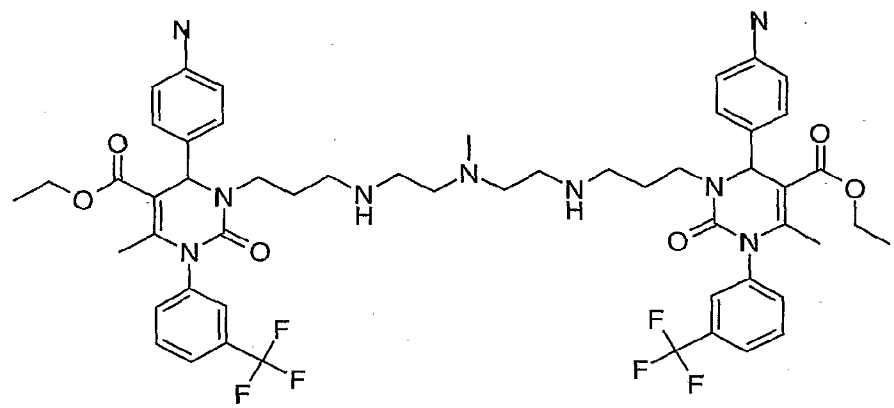


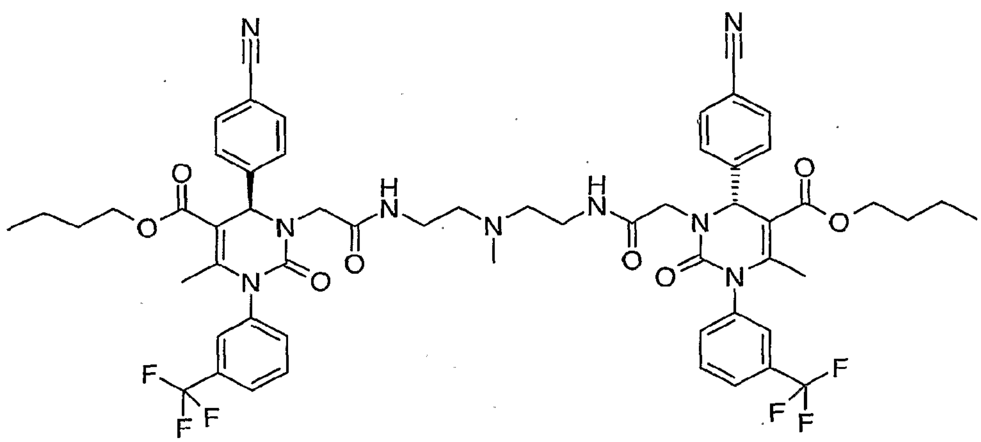


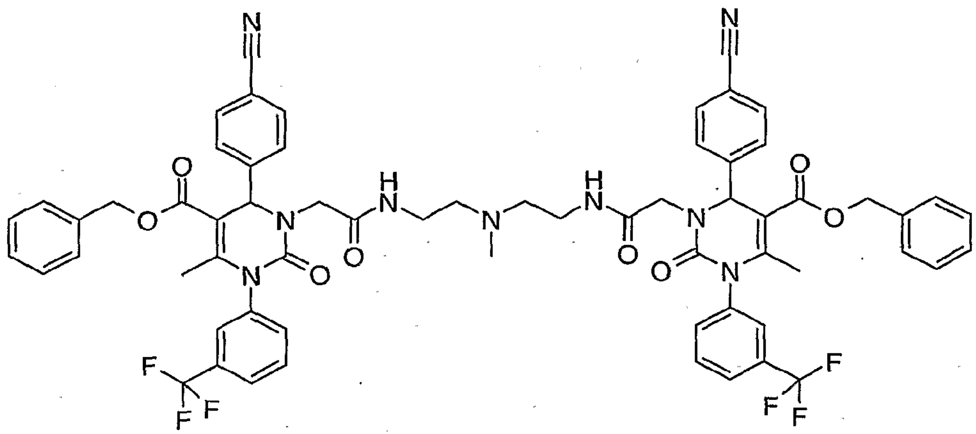


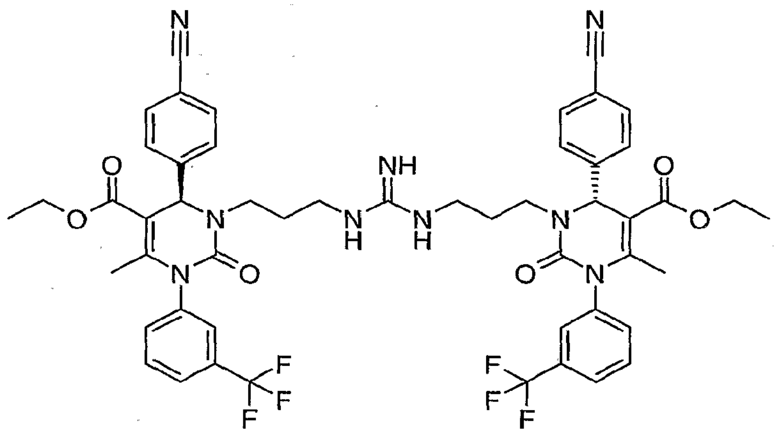


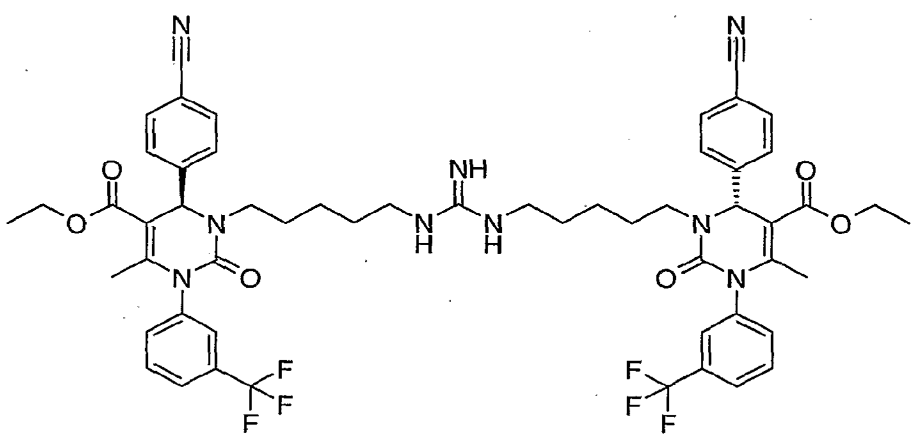


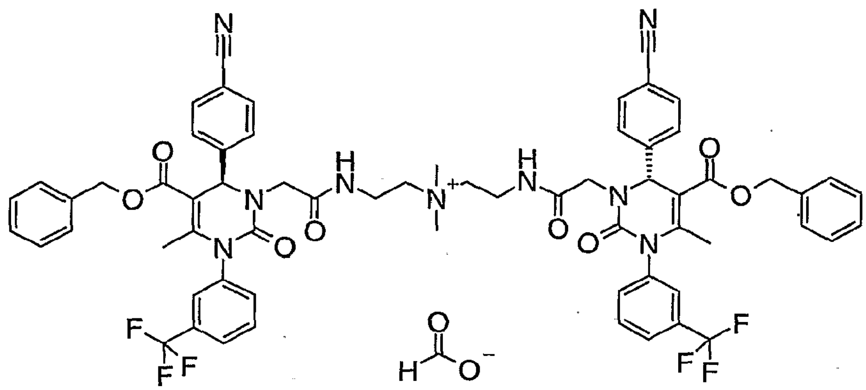


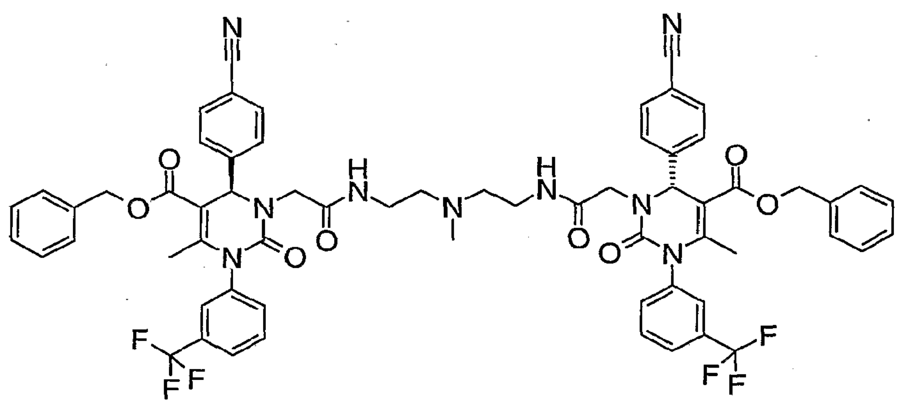


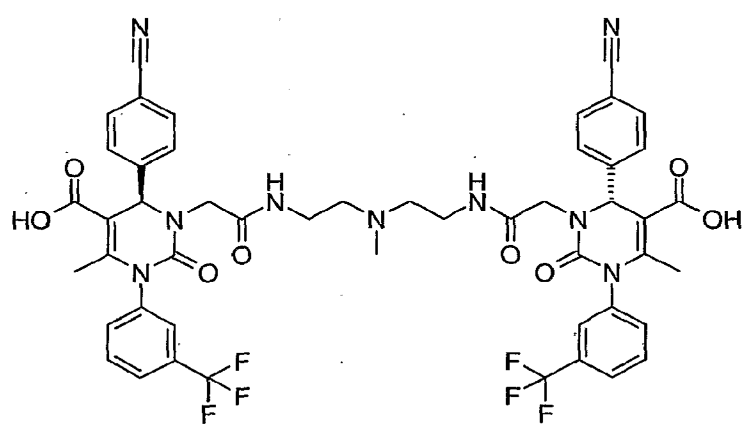

N
O
O
N
N
O
H
N
O
N
N
H
N
O
N
N
O
O
O
N
F
F
F
F
F
F

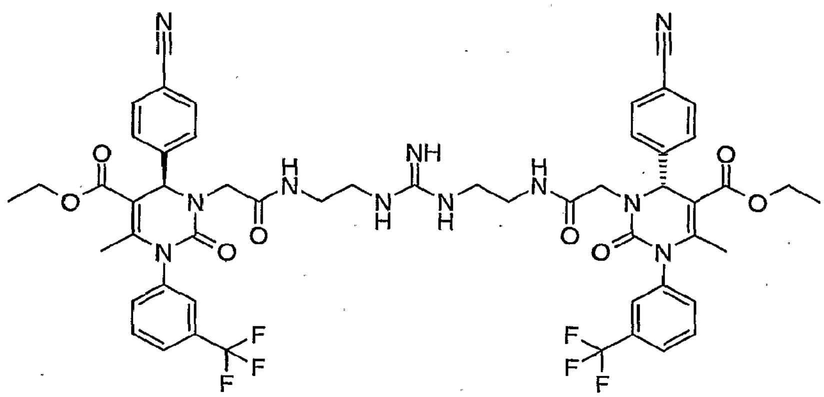

N
N
NH
O
O
N
N
O
N
H
N
H
N
N
O
O
O
F
F
F
F
F
F

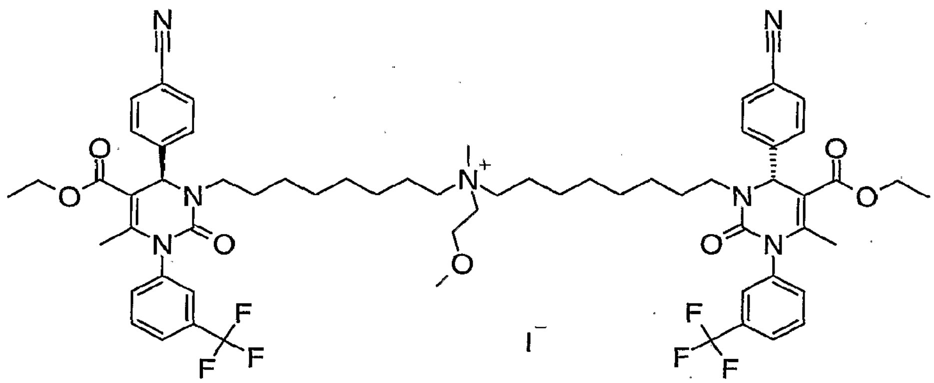

N
N
NH
O
O
N
N
O
N
H
N
H
N
N
O
O
O
F
F
F
F
F
F

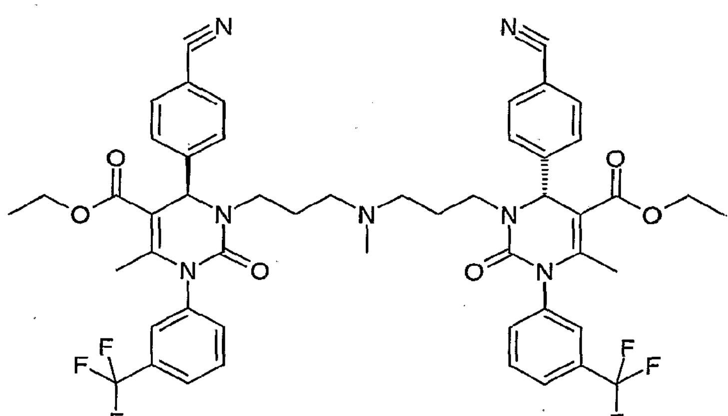


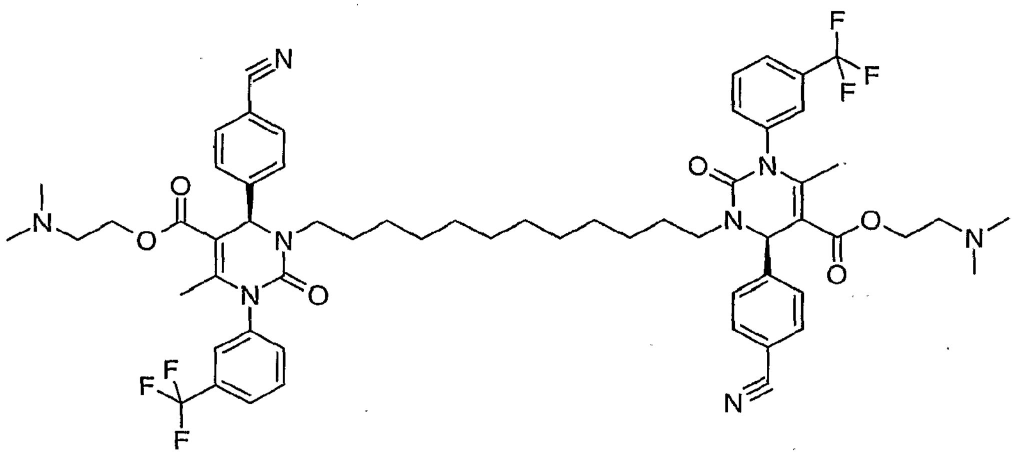


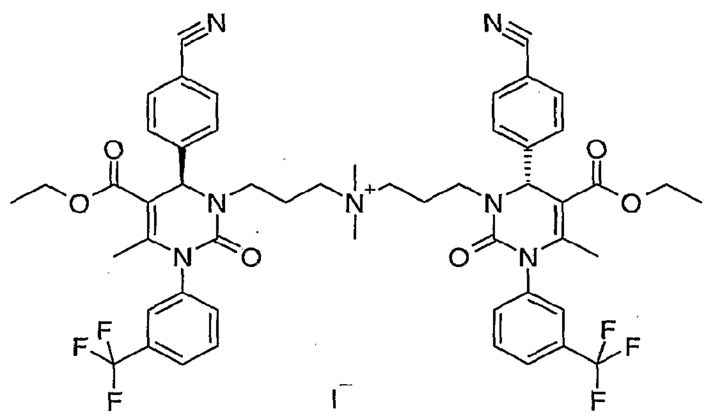


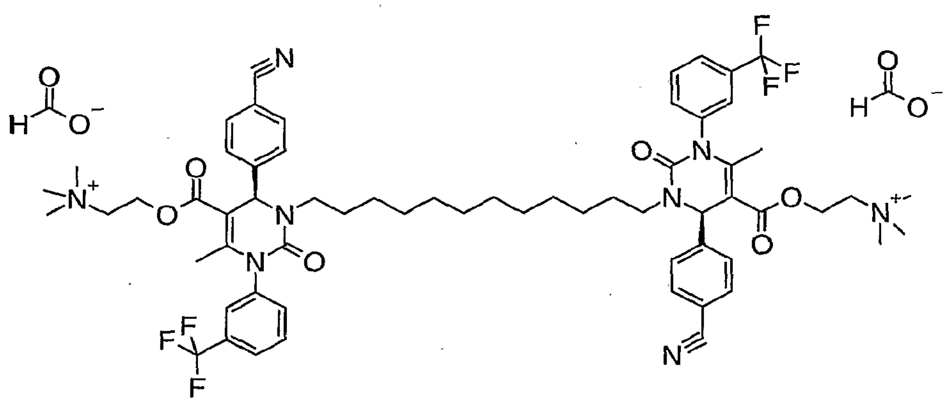


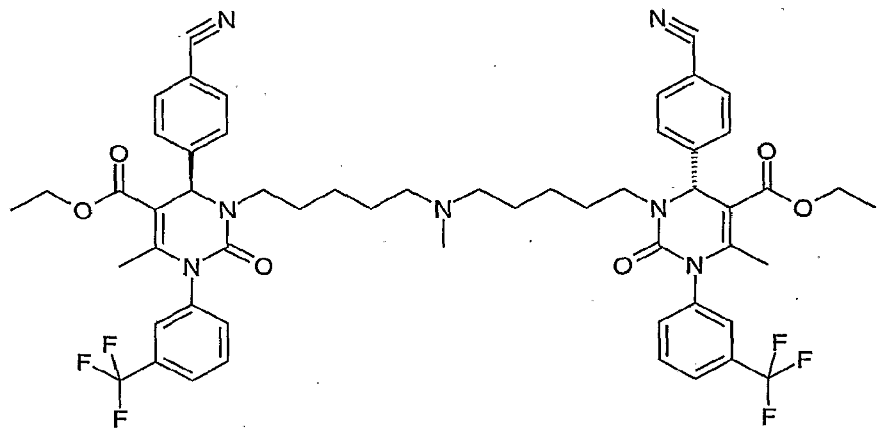


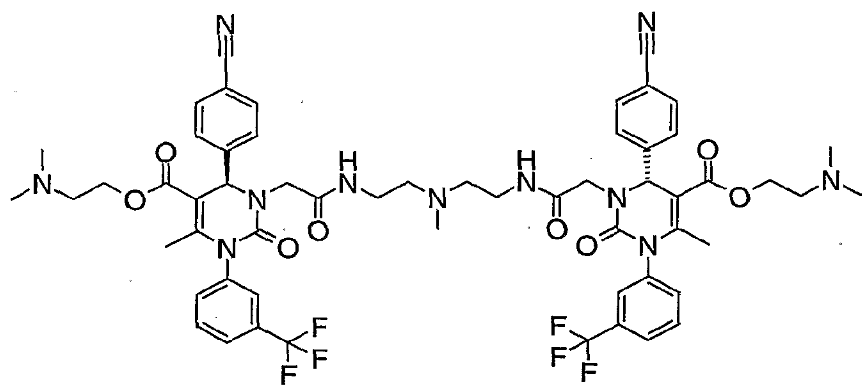


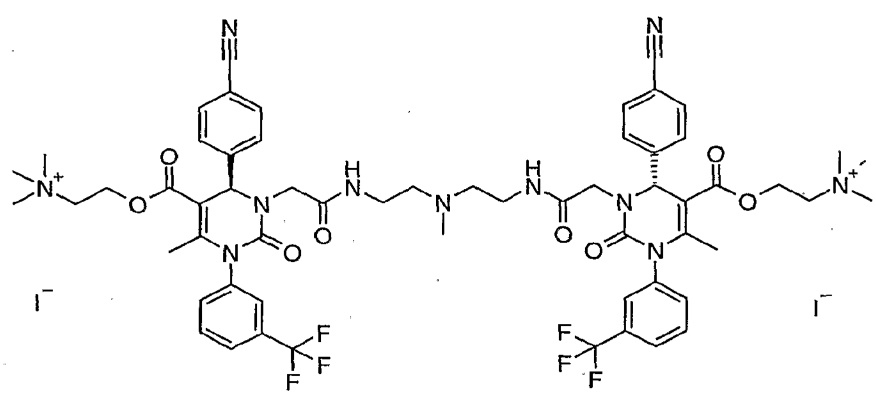


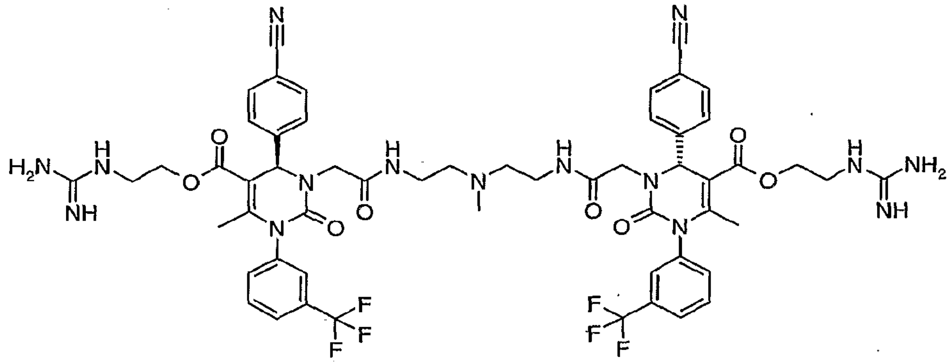


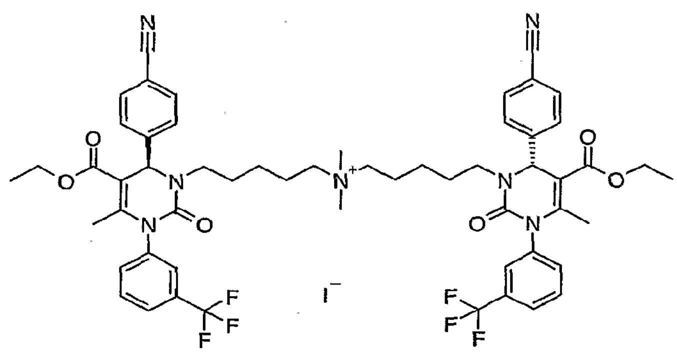


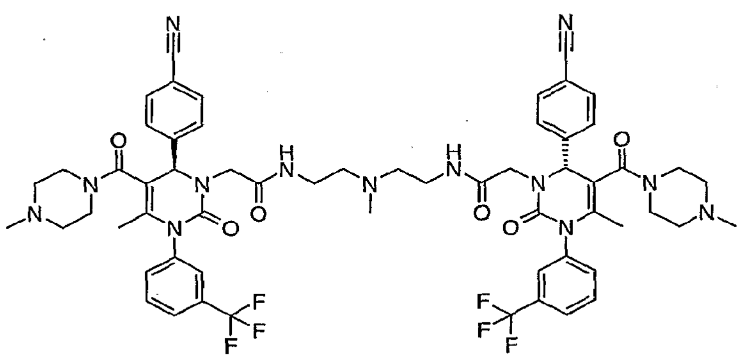


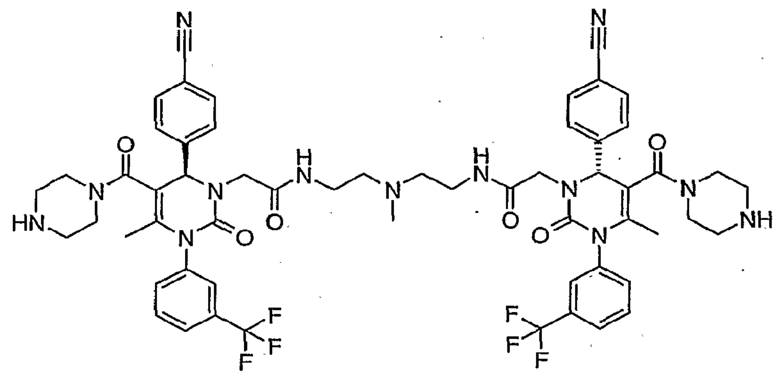


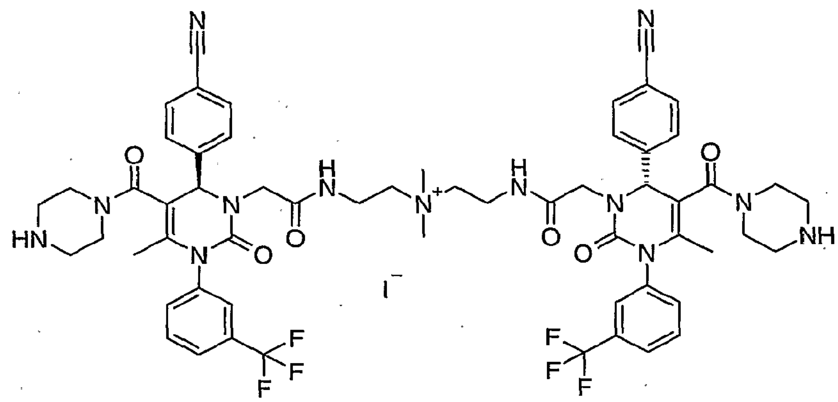


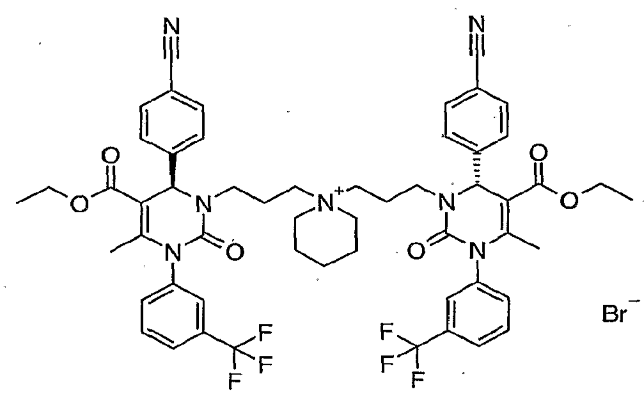


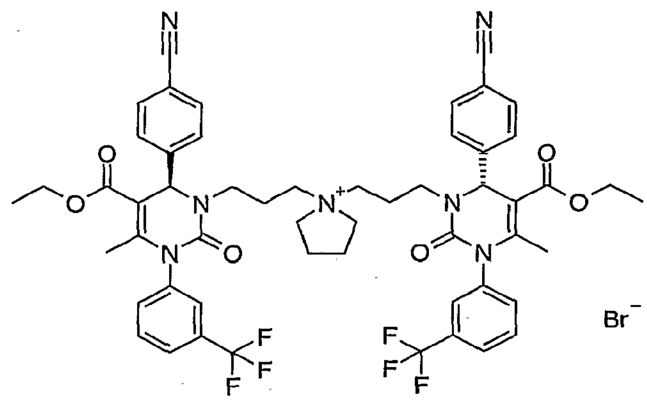


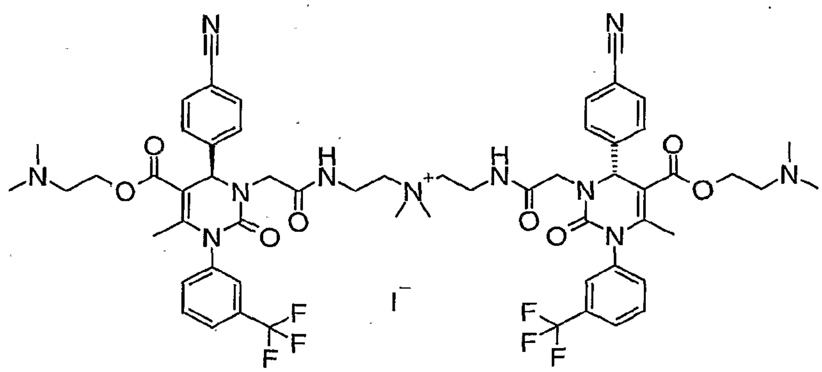


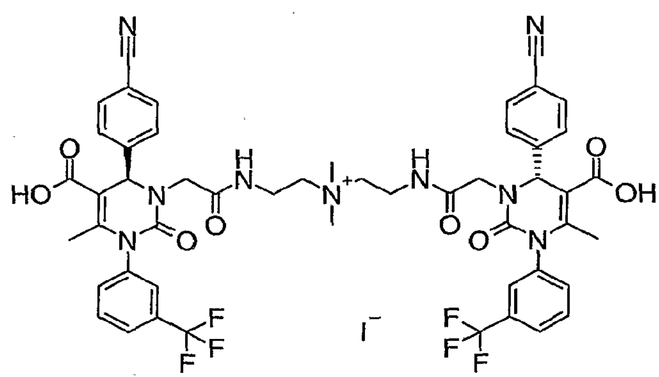


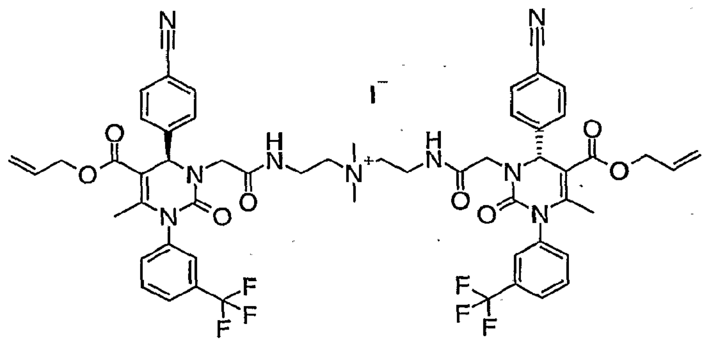












The therapeutic utility of the present compounds is pertinent to any disease that is known to be at least partially mediated by the action of human neutrophil elastase. For example, the present compounds may be beneficial in the treatment of chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), pulmonary emphysema, pneumonia and lung fibrosis.

The present invention is also concerned with pharmaceutical formulations comprising, as an active ingredient, a compound of the invention. Other compounds may be combined with compounds of this invention for the prevention and treatment of inflammatory diseases of the lung. Thus the present invention is also concerned with pharmaceutical compositions for preventing and treating inflammatory diseases of the lung comprising a therapeutically effective amount of a compound of the invention and one or more other therapeutic agents.

Suitable therapeutic agents for a combination therapy with compounds of the invention include: (1) a corticosteroid, for example fluticasone, cilomilast or budesonide; (2) a β2-adrenoreceptor agonist, for example salmeterol or formeterol; (3) a leukotriene modulator, for example montelukast or pranlukast; (4) muscarinic-3 (M3) receptor antagonists such as tiotropium bromide; (5) bronchodilators that possess both M3 receptor antagonism and β2-adrenoreceptor agonism in a single molecule (6) phosphodiesterase-IV (PDE-IV) inhibitors, for example roflumilast or cilomilast; (7) an antitussive agent, such as codeine or dextramorphan;(8) a non-steroidal anti-inflammatory agent (NSAID), for example ibuprofen or ketoprofen; (9) kinase inhibitors such as p38 MAP kinase inhibitors, IKK2 inhibitors and (10) receptor antagonists for cytokines and chemokines for example IL-8, MCP-1, TNFα and IL-1β.

The weight ratio of the first and second active ingredients may be varied and will depend upon the effective dose of each ingredient. Generally, an effective dose of each will be used.

The magnitude of prophylactic or therapeutic dose of a compound of the invention

will, of course, vary with the nature of the severity of the condition to be treated and with the particular compound and its route of administration. It will also vary according to the age, weight and response of the individual patient. In general, the daily dose range will lie within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be necessary to use dosages outside these limits in some cases.

Another aspect of the present invention provides pharmaceutical compositions which comprise a compound of the invention and a pharmaceutically acceptable carrier. The term “composition”, as in pharmaceutical composition, is intended to encompass a product comprising the active ingredient(s), and the inert ingredient(s) (pharmaceutically acceptable excipients) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the invention, additional active ingredient(s), and pharmaceutically acceptable excipients.

The pharmaceutical compositions of the present invention comprise a compound of the invention as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term “pharmaceutically acceptable salts” refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic bases or acids and organic bases or acids.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound of the present invention. In therapeutic use, the active compound may be administered by any convenient, suitable or effective route. Suitable routes of administration are known to those skilled in the art, and include oral, intravenous, rectal, parenteral, topical, ocular, nasal, buccal and pulmonary. Delivery by inhalation is preferred.

Compositions suitable for administration by inhalation are known, and may include carriers and/or diluents that are known for use in such compositions. The composition may contain 0.01-99% by weight of active compound. Preferably, a unit dose comprises the active compound in an amount of 1μg to 10 mg.

The most suitable dosage level may be determined by any suitable method known to one skilled in the art. It will be understood, however, that the specific amount for any particular patient will depend upon a variety of factors, including the activity of the

specific compound that is used, the age, body weight, diet, general health and sex of the patient, time of administration, the route of administration, the rate of excretion, the use of any other drugs, and the severity of the disease undergoing treatment.

For delivery by inhalation, the active compound is preferably in the form of microparticles. They may be prepared by a variety of techniques, including spray-drying, freeze-drying and micronisation.

By way of example, a composition of the invention may be prepared as a suspension for delivery from a nebuliser or as an aerosol in a liquid propellant, for example for use in a pressurised metered dose inhaler (PMDI). Propellants suitable for use in a PMDI are known to the skilled person, and include CFC-12, HFA-134a, HFA-227, HCFC-22 (CCl2F2) and HFA-152 (CH4F2 and isobutane).

In a preferred embodiment of the invention, a composition of the invention is in dry powder form, for delivery using a dry powder inhaler (DPI). Many types of DPI are known.

Microparticles for delivery by administration may be formulated with excipients that aid delivery and release. For example, in a dry powder formulation, microparticles may be formulated with large carrier particles that aid flow from the DPI into the lung. Suitable carrier particles are known, and include lactose particles; they may have a mass median aerodynamic diameter of greater than 90 μm.

In the case of an aerosol-based formulation, a preferred composition is:

Compound of the invention	24 mg / canister
Lecithin, NF Liq. Conc.	1.2 mg / canister
Trichlorofluoromethane, NF	4.025 g / canister
Dichlorodifluoromethane, NF	12.15 g / canister.

Compounds of the invention may be used in combination with other drugs that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which present compounds are useful. Such other drugs may be administered, by a route and in an amount commonly used therefore, contemporaneously or sequentially with a compound of the invention. When a compound of the invention is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of the invention is preferred. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to a compound of the invention.

The agents of the invention may be administered in inhaled form. Aerosol generation can be carried out using, for example, pressure-driven jet atomizers or ultrasonic atomizers, preferably using propellant-driven metered aerosols or propellant-

free administration of micronized active compounds from, for example, inhalation capsules or other "dry powder" delivery systems.

The active compounds may be dosed as described depending on the inhaler system used. In addition to the active compounds, the administration forms may additionally contain excipients, such as, for example, propellants (e.g. Frigen in the case of metered aerosols), surface-active substances, emulsifiers, stabilizers, preservatives, flavorings, fillers (e.g. lactose in the case of powder inhalers) or, if appropriate, further active compounds.

For the purposes of inhalation, a large number of systems are available with which aerosols of optimum particle size can be generated and administered, using an inhalation technique which is appropriate for the patient. In addition to the use of adaptors (spacers, expanders) and pear-shaped containers (e.g. Nebulator®, Volumatic®), and automatic devices emitting a puffer spray (Autohaler®), for metered aerosols, in particular in the case of powder inhalers, a number of technical solutions are available (e.g. Diskhaler®, Rotadisk®, Turbohaler® or the inhalers for example as described EP-A-0505321).

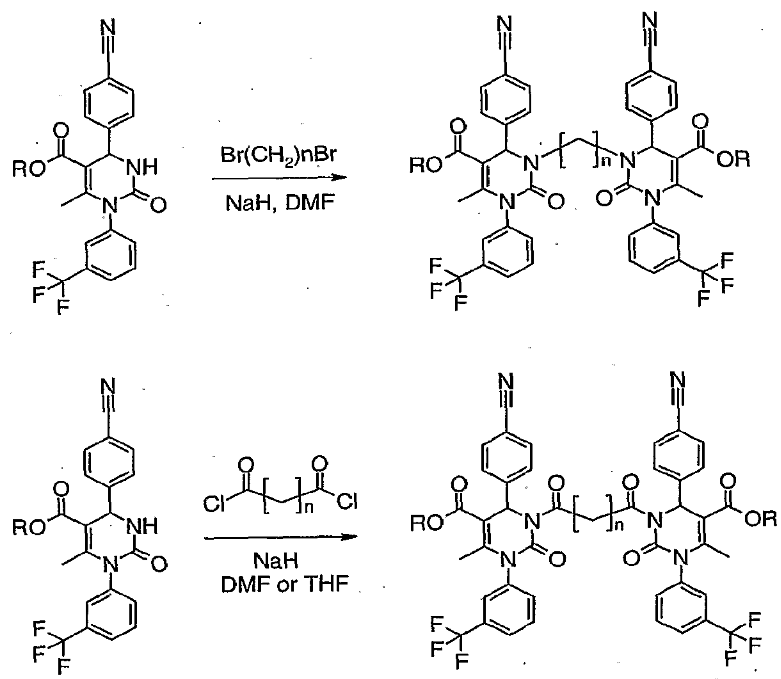
The compounds of the invention of the present invention can be prepared according to the procedures of the following schemes and examples, using appropriate materials, and are further exemplified by the following specific examples. Moreover, by utilising the procedures described with the disclosure contained herein, one of ordinary skill in the art can readily prepare additional compounds of the present invention claimed herein. The compounds illustrated in the examples are not, however, to be construed as forming the only genus that is considered as the invention. The examples further illustrate details for the preparation of the compounds of the present invention. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds.

The compounds of the invention may be isolated in the form of their pharmaceutically acceptable salts, such as those described previously herein above. The free acid form corresponding to isolated salts can be generated by neutralisation with a suitable acid such as acetic acid and hydrochloric acid and extraction of the liberated free acid into an organic solvent followed by evaporation. The free acid form isolated in this manner can be further converted into another pharmaceutically acceptable salt by dissolution in an organic solvent followed by addition of the appropriate base and subsequent evaporation, precipitation, or crystallisation.

It may be necessary to protect reactive functional groups (e.g. hydroxy, amino, thio or carboxy) in intermediates used in the preparation of compounds of the invention to

avoid their unwanted participation in a reaction leading to the formation of the compounds. Conventional protecting groups, for example those described by T. W. Greene and P. G. M. Wuts in "Protective groups in organic chemistry" John Wiley and Sons, 1999, may be used.

The following reaction schemes illustrate how compounds of the invention, in particular the Example compounds may be prepared. It will be understood that the processes detailed below are solely for the purpose of illustrating the invention and should not be construed as limiting. A process utilising similar or analogous reagents and/or conditions known to one skilled in the art may also be used to obtain a compound of the invention.

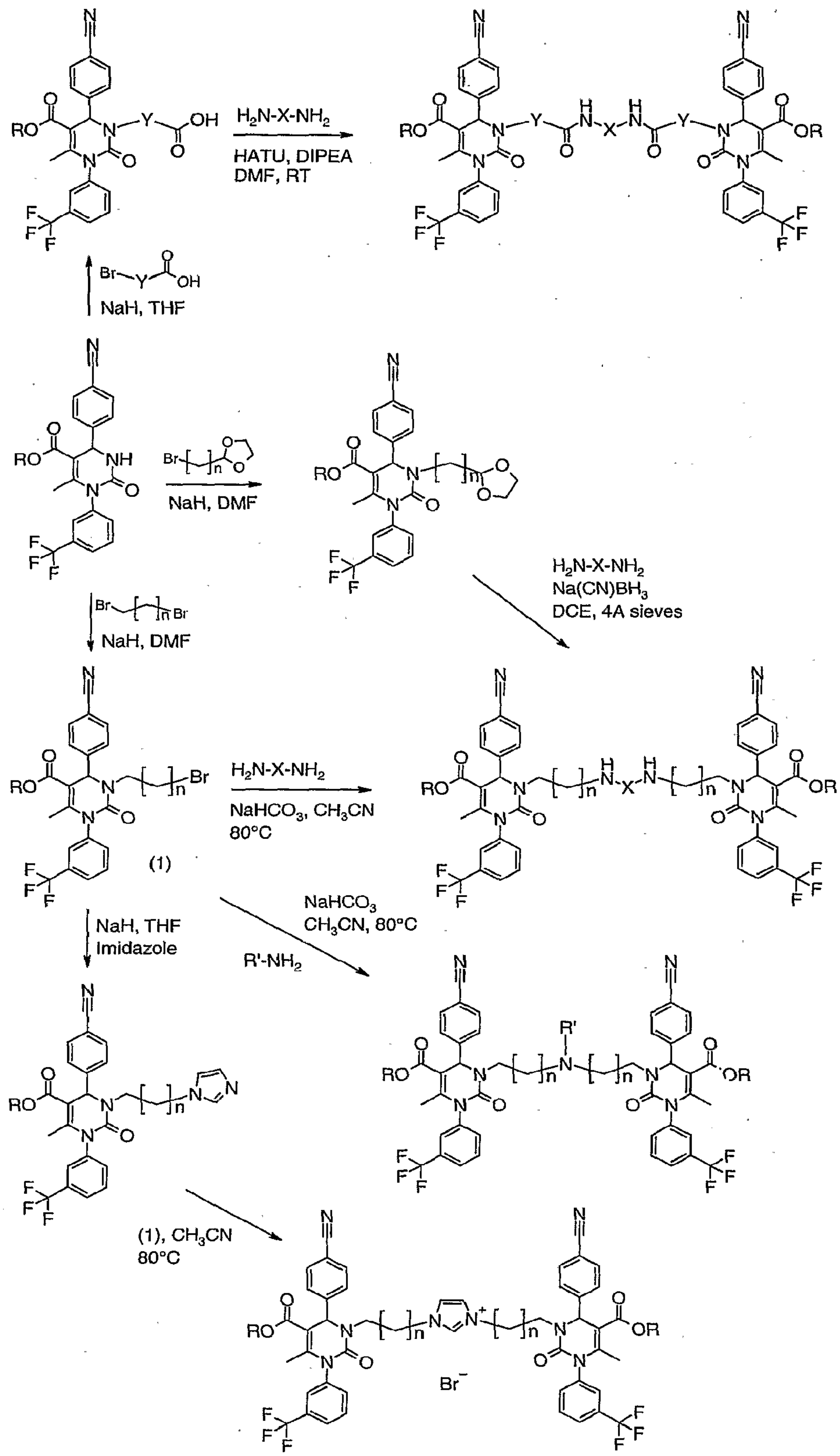


Scheme 1

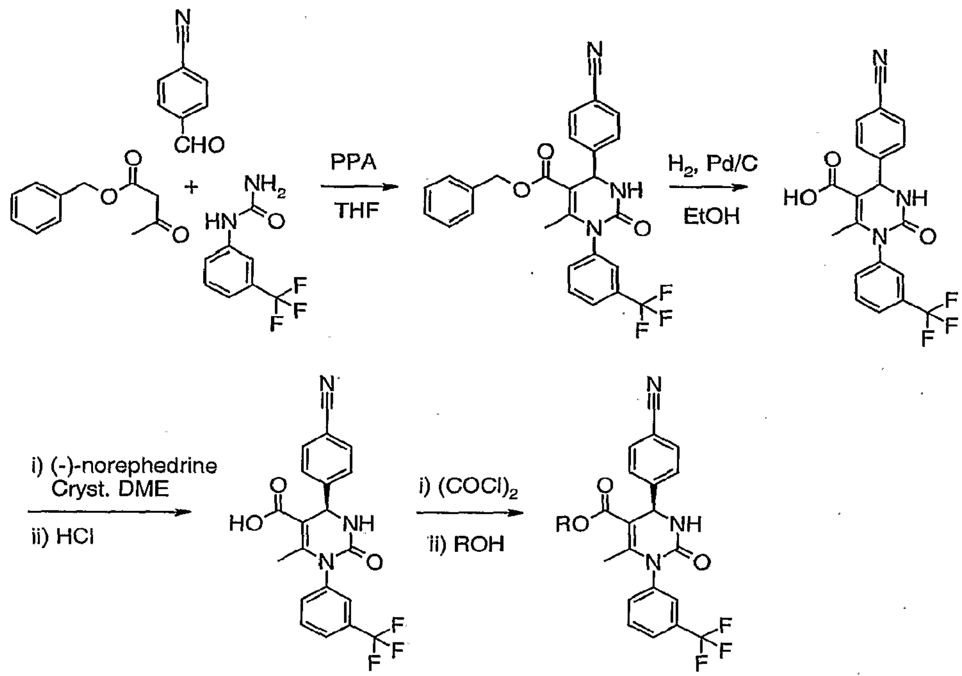
The monomers may be joined together by a range of standard chemistries. For example, alkylation with a suitable $\alpha\omega$-difunctionalised alkane can be effected in the presence of a suitable base, for example a metal hydride and optional solvent. Suitable leaving groups include halogen and sulfonate. Similarly, monomers may be joined by bis-acylation with a bis acid halide under similar conditions, Scheme 1.

An alternative methodology involves attachment of a spacer group incorporating a second functional group, which subsequently allows attachment of a bidenetate linker

group, as in Scheme 2. Alkylation of the monomer with a suitably substituted ω-halo acid (protected as an ester), ω-haloaldehyde (protected as an acetal), or αω-dihaloalkane, employing a base such as NaH in a suitable inert solvent such as DMF or THF, followed by deprotection as necessary leads to intermediates that can be reacted with suitable bidentate species to generate compounds of the invention, Scheme 2.



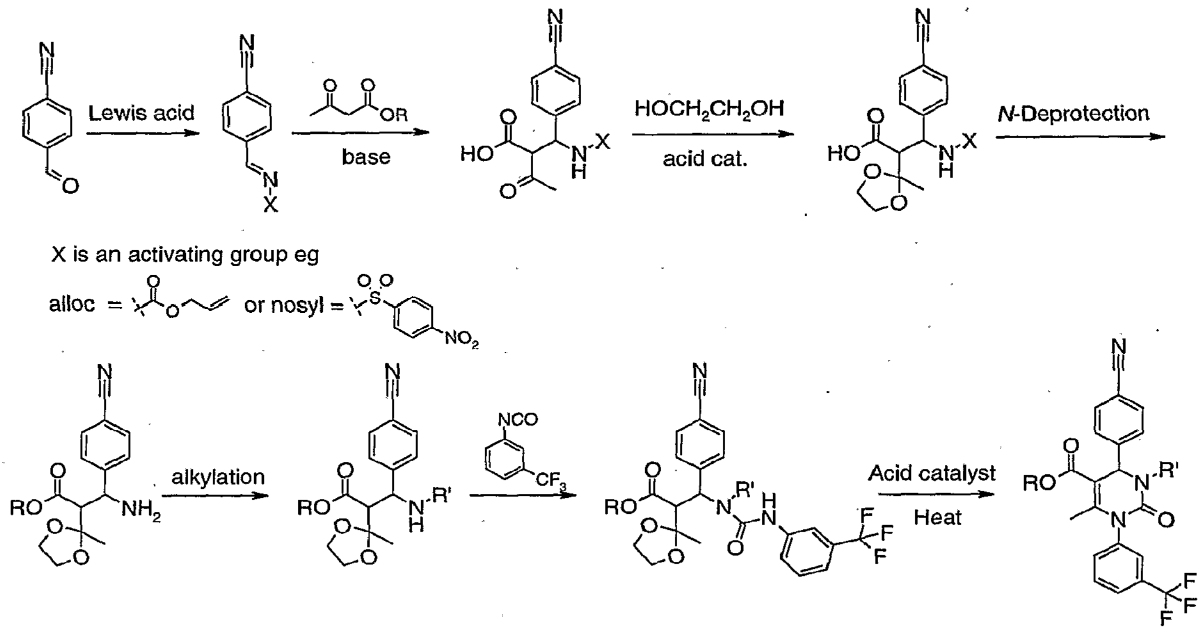
Scheme 2



Scheme 3

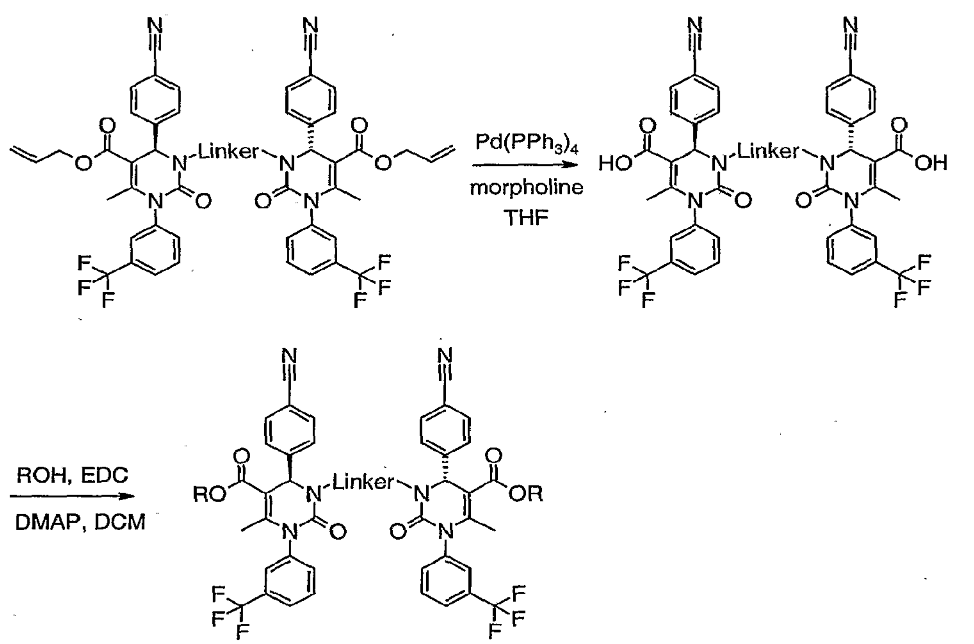
The monomers of Formula (II) may be prepared as racemates according to methods described in WO2004024700, WO2004024701, GB2392910, WO200508263 and WO2005082864. The monomers may be separated by chiral HPLC into their enantiomers.

Alternatively the monomer (as a carboxylic acid) may be resolved by formation of diastereomeric salts with a suitable chiral base, such as norephedrine, followed by fractional recrystallisation, Scheme 3.



Scheme 4

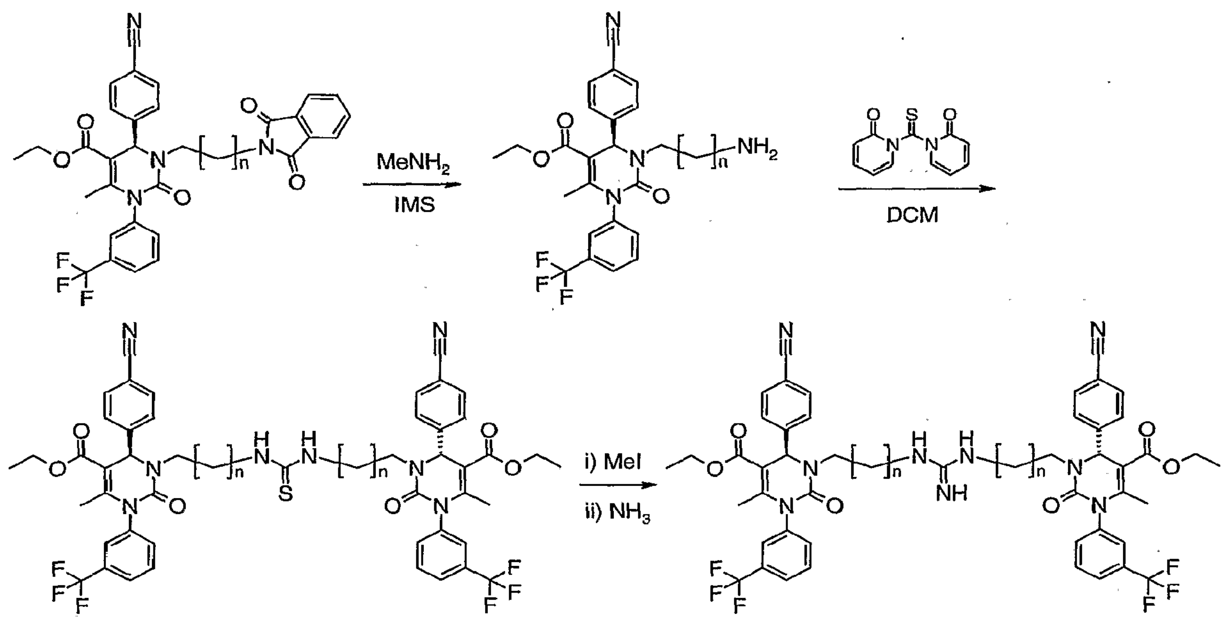
An alternative method for the preparation of compounds of Formula (II) is illustrated in Scheme 4.



Scheme 5

In some cases the ester corresponding to R^4 of compounds of Formula (II) may be hydrolysed to a parent acid using suitable conditions, and then interconverted to other esters using standard ester coupling conditions. Additionally, the acid may be converted

to amides and other compounds of the invention using standard methods familiar to those skilled in the art, Scheme 5.



Scheme 6

Compounds of Formula (II) containing a guanidine moiety in the linker group may be prepared according to the method outlined in Scheme 6.

Compounds that are quaternary ammonium salts generally have counter-ions that are derived from the alkylating agent used in the quaternisation, i.e reaction of a tertiary amine with methyl iodide leads to a quaternary ammonium iodide. This counter-ion can be exchanged to another counter-ion according to the method of Bach Nielsen, Frydenvang, Liljeforts, Buur & Larsen, Eur. J. Pharm. Sci, 24 (2005) 85-93. Compounds containing alternative counter-ions may be expected to have different

General Experimental Details:

All reactions were carried out under an inert atmosphere of nitrogen unless specified otherwise.

Where products were purified by column chromatography on silica, 'silica' refers to silica gel for chromatography, 0.035 to 0.070 mm (220 to 440 mesh) (e.g. Fluka silica gel 60), and an applied pressure of nitrogen up to 10 p.s.i for accelerated column elution. All solvents and commercial reagents were used as received.

Preparative HPLC conditions:

HPLC system 1:

C18-reverse-phase column (100 × 22.5 mm i.d Genesis column with 7 μm particle size), eluting with a gradient of A: water + 0.1% TFA; B: acetonitrile + 0.1 % TFA at a flow rate of 5 ml/min and gradient of 1 %/min increasing in B. UV detection at 230 nm.

HPLC system 2:

Phenyl hexyl column (250 × 21.20 mm Luna column with 10 μm particle size), eluting with an isocratic mixture of A: water + 0.1% TFA; B: acetonitrile + 0.1 % TFA at a flow rate of 20 ml/min with UV detection at 254 nm.

HPLC system 3:

C18-reverse-phase column (100 × 22.5 mm i.d Genesis column with 7 μm particle size), eluting with a gradient of A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid at a flow rate of 5 ml/min and gradient of 1 %/min increasing in B. UV detection at 230 nm.

HPLC system 4:

C18-reverse-phase column (100 × 22.5 mm i.d Genesis column with 7 μm particle size), eluting with a gradient of A: water + 0.1% formic acid; B: MeOH + 0.1 % formic acid at a flow rate of 5 ml/min and gradient of 1 %/min increasing in B. UV detection at 230 nm.

HPLC system 5:

Phenyl hexyl column (250 × 21.20 mm Luna column with 10 μm particle size), eluting with an isocratic mixture of A: water + 0.1% TFA; B: MeOH + 0.1 % TFA at a flow rate of 20 ml/min with UV detection at 254 nm.

After HPLC purification, fractions containing product were combined and freeze-dried to give the product as a white or off-white solid. In some cases, where the compound contained a basic centre, the product was obtained as the TFA or formate salt.

LC/MS Systems

The Liquid Chromatography Mass Spectroscopy (LC/MS) systems used:

LC-MS method 1

Micromass Platform LCT with a C18-reverse-phase column (100 × 3.0 mm Higgins Clipeus with 5 μm particle size), elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient – Time	flow ml/min	%A	%B
0.00	1.0	95	5
1.00	1.0	95	5
15.00	1.0	5	95
20.00	1.0	5	95
22.00	1.0	95	5
25.00	1.0	95	5

Detection - MS, ELS, UV (100 μl split to MS with in-line UV detector)
MS ionisation method - Electrospray (positive ion)

LC-MS method 2

Micromass Platform LCT with a C18-reverse-phase column (30 × 4.6 mm Phenomenex Luna 3 μm particle size), elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient – Time	flow ml/min	%A	%B
0.00	2.0	95	5
0.50	2.0	95	5
4.50	2.0	5	95
5.50	2.0	5	95
6.00	2.0	95	5

Detection - MS, ELS, UV (100 μl split to MS with in-line UV detector)
MS ionisation method - Electrospray (positive and negative ion)

LC-MS method 3

Waters Micromass ZQ with a C18-reverse-phase column (30 × 4.6 mm Phenomenex Luna 3 μm particle size), elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient – Time	flow ml/min	%A	%B

0.00	2.0	95	5
0.50	2.0	95	5
4.50	2.0	5	95
5.50	2.0	5	95
6.00	2.0	95	5

Detection - MS, ELS, UV (100 µl split to MS with in-line UV detector)
MS ionisation method - Electrospray (positive and negative ion)

<u>LC-MS method 4</u>

Waters Micromass ZMD with a C18-reverse-phase column (30 × 4.6 mm Phenomenex Luna 3 µm particle size), elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient – Time	flow ml/min	%A	%B
0.00	2.0	95	5
0.50	2.0	95	5
4.50	2.0	5	95
5.50	2.0	5	95
6.00	2.0	95	5

Detection - MS, ELS, UV (100 µl split to MS with in-line UV detector)
MS ionisation method - Electrospray (positive and negative ion)

NMR Spectrometers

NMRs were run on either a Varian Unity Inova 400 MHz spectrometer or a Bruker Avance DRX 400 MHz spectrometer.

Abbreviations used in the experimental section:

DCM = dichloromethane
THF = tetrahydofuran
DMF = *N,N*-dimethylformamide
DIPEA = di-isopropylethylamine
RT = room temperature
HATU = *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-

tetramethyluroniumhexafluorophosphate.

TFA = trifluoroacetic acid

Boc = tertiary-butyloxycarbonyl

Rt = retention time

EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride

DCE = 1,1-dichloroethane

IPA = isopropyl alcohol

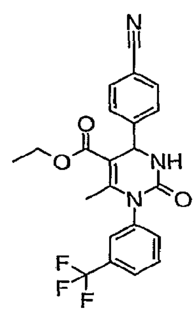
IMS = industrial methylated spirits

EtOAc = ethyl acetate

MPLC = Medium pressure liquid chromatography

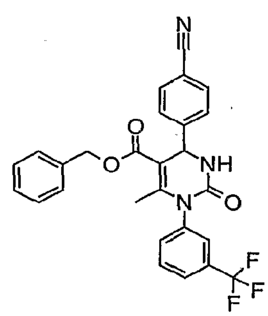
DMAP = 4-N,N-dimethylaminopyridine

Intermediate 1



Intermediate 1 was prepared according to WO2004/024700.

Intermediate 2

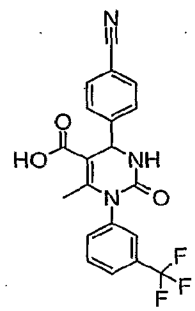


Polyphosphoric acid (350 g) was suspended in THF (1.9 l) and stirred, mechanically, whilst 3-(trifluoromethyl)phenylurea (129 g), 4-cyanobenzaldehyde (100 g) and benzyl acetoacetate (121.3 g) were added. The resulting mixture was heated at reflux overnight. The bulk of the solvent was evaporated under reduced pressure and the residue partitioned between water and EtOAc. The organic phase was washed with water, aqueous K_2CO_3 solution, water, brine, dried (Na_2SO_4) and evaporated. The residue was triturated with diethyl ether to give Intermediate 2 as a yellow solid.

Yield: 216.6g (70%)

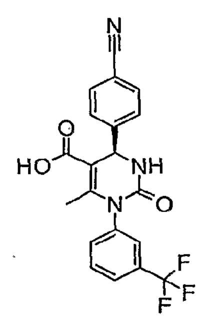
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.38 (1H, d), 7.84 (2H, m), 7.80-7.55 (6H m), 7.30 (3H, m), 7.15 (2H, m), 5.13 (1H, d), 5.06 (1H, d), 5.39 (1H, d), 2.08 (3H, s).

Intermediate 3



Intermediate 2 (215 g, 0.54 mol) was suspended in EtOAc (1.5 l) and IMS (500 ml) was added. The mixture was warmed slightly by immersion in a warm water bath (ca. 40ºC) until homogeneous and then treated, under nitrogen, with $Pd(OH)_2$ on carbon (20 g, 20% w/w, 50% water wet). The flask was sealed and evacuated and then the mixture was stirred under an atmosphere of hydrogen whilst keeping warm by replenishment of the warm water bath. After ca. 4-6 h, the flask was evacuated and the mixture filtered through 'hyflo'. The filtrate was evaporated under vacuum and the residue triturated with diethyl ether to furnish Intermediate 3 as a solid which was used directly in the next step.

Intermediate 4

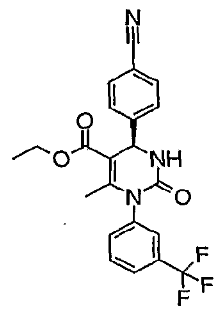


Intermediate 3 (156.2 g) was suspended in IPA (1500 ml) and treated with L-(-)-norephedrine. The mixture became homogeneous and then began to deposit a solid. After stirring for ca. 6 h the solid was filtered off and washed with IPA and sucked as dry as possible on the filter. The cake was removed and dissolved in a minimum amount of hot IPA (ca. 2.5 l) and allowed to cool, with stirring, overnight. The mixture was filtered and washed with IPA and dried to give a white solid. This was partitioned between EtOAc and 2M HCl until all solid had dissolved. The organic layer was washed with brine, dried (Na_2SO_4) and evaporated to give Intermediate 4 as a colourless foam (61.7 g).

Yield: 61.7 g (29%)

^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 12.48 (1H, br s), 8.32 (1H, d), 7.88 (2H, m), 7.79-7.54 (6H, m), 5.36 (1H, d), 4.03 (2H, q), 2.05 (3H, s), 1.18 (3H, t).

Intermediate 5

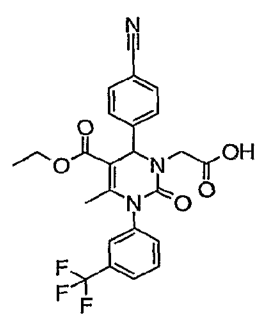


Intermediate 4 (47.6 g) was suspended in toluene and treated with oxalyl chloride (18 g; 12.5 ml). The mixture was stirred and treated with DMF (20 drops). The resulting mixture was stirred at room temperature until gas evolution ceased and the mixture was homogeneous (ca. 1h). The solution was cooled and treated with diisopropylethylamine (20 ml) followed by a mixture of EtOH (20 ml) and diisopropylethylamine (20 ml). After stirring at room temperature for ca 30 min, the solution was diluted with EtOAc and washed with 2M HCl, water, aqueous K_2CO_3, water, brine, dried (Na_2SO_4) and evaporated to a brown foam. Chromatography on silica gel, eluting with 0-20% EtOAc in DCM as eluant, gave Intermediate 5 as a pale yellow foam.

Yield: 40.3 g (79%)

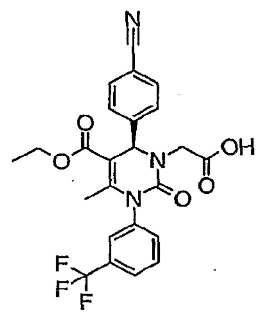
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.72-7.39 (8H, m), 5.95 (1H, d), 5.58 (1H, d), 4.15 (2H, q), 2.10 (3H, s), 1.21 (3H, t).

Intermediate 6



Intermediate 6 was prepared from Intermediate 1 according to WO2004/024700.

Intermediate 7

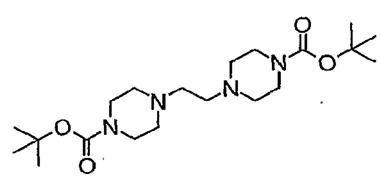


Intermediate 5 (1.69 g, 3.94 mmol) was dissolved in DMF (35 ml) and the solution was cooled to −10ºC under argon. Sodium hydride (60% dispersion in mineral oil) (394 mg, 9.85 mmol) was added and the reaction was stirred for 10 min before the addition of bromoacetic acid. Stirring was continued for 1.5 h and then the reaction was quenched with sat. NH_4Cl (10 ml). The mixture was diluted with 1M HCl (100 ml) and extracted with EtOAc (3 × 75 ml). The combined organic extracts were washed with water (100 ml) and brine (50 ml), dried (Na_2SO_4) and evaporated. The residue was taken up into acetonitrile (20 ml) and loaded onto an Isolute NH_2 cartridge (25 g). The cartridge was flushed with acetonitrile (250 ml) and then the product was eluted with 2% conc. aqueous HCl in acetonitrile (350 ml). The fractions containing the product were combined and the volume reduced. The residue was partitioned between EtOAc (150 ml) and 1M aqueous HCl (75 ml). The organic layer was separated, washed with water (70 ml) and brine (50 ml), dried (Na_2SO_4) and evaporated. The product was obtained as a pale cream foam.

Yield: 1.63 g (85%)

LC-MS (Method 2): Rt 3.67 min, m/z 488 [MH^+]

Intermediate 8

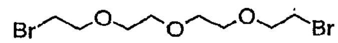


Boc-Piperazine (0.5 g, 2.69 mmol), 1,2-dibromoethane (253 mg, 1.34 mmol) and $NaHCO_3$ (564 mg, 6.72 mmol) in acetonitrile (20 ml) were heated at 90°C for 17h. After cooling to RT, the solvent was removed and the residue was dissolved in EtOAc (80 ml). The organic solution was washed with water (50 ml) and brine (20 ml), dried (Na_2SO_4) and evaporated to give Intermediate 8 as a white solid.

Yield: 426 mg (80%)

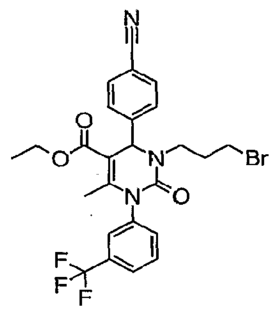
LC-MS (Method 2): Rt 0.34/2.05 min, m/z 399 [MH^+]

Intermediate 9



Prepared according to: Mills, O. S.; Mooney, N. J.; Robinson, P. M.; Watt, C. I. F.; Box, B. G. J. Chem. Soc., Perkin Trans. 2: (1995) 4, 697-706.

Intermediate 10

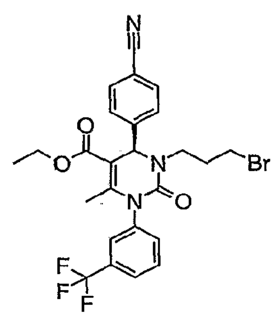


Intermediate 1 (2.00 g, 4.66 mmol) was dissolved in anhydrous DMF (40 ml) and the solution was cooled to 0°C. Sodium hydride (60% dispersion in mineral oil) (280 mg, 7.00 mmol) was added and the reaction mixture was stirred until the effervescence had ceased. 1,3-Dibromopropane (2.5 ml) was added and stirring was continued at 0°C for a further 1 h, after which time 1M HCl (60 ml) and diethyl ether (60 ml) were added. The organic layer was separated and the aqueous solution was further extracted with diethyl ether (2 × 50 ml). The organic extracts were combined and washed with water (50 ml) and brine (30 ml), dried (Na_2SO_4) and evaporated. The residue was purified using MPLC on silica gel eluting with 20% EtOAc in pentane.

Yield: 1.05 g (41%)

LC-MS (Method 3): Rt 4.12 min, m/z 550/552 [MH^+]

Intermediate 11

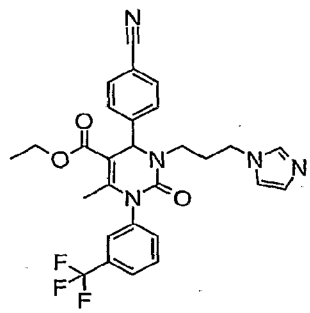


Intermediate 11 was prepared in a similar manner from Intermediate 5. The product was purified on silica eluting with DCM.

Yield: 65%

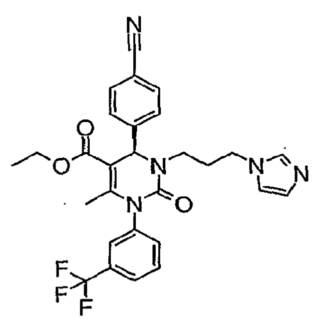
LC-MS (Method 3): Rt 4.10 min, m/z 550/552 [MH^+]

Intermediate 12



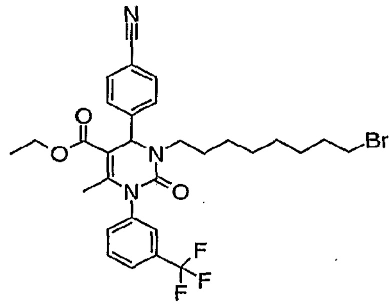
Imidazole (12 mg, 0.182 mmol) was dissolved in anhydrous THF (10 ml) and sodium hydride (60% dispersion in mineral oil) (7 mg, 0.175 mmol) was added. The reaction mixture was stirred until the effervescence had ceased and Intermediate 10 (200 mg, 0.364 mmol) was added. The reaction mixture was heated at 80°C for 40 h, after which time it was allowed to cool and the solvent was evaporated. EtOAc (40 ml) and water (40 ml) were added and the organic layer was separated. The aqueous solution was further extracted with EtOAc (2 × 50 ml) and the organic extracts were combined, washed with water (50 ml) and brine (30 ml), dried (Na_2SO_4) and evaporated. The residue was purified using an Isolute Si(II) cartridge (10 g) eluting with 0-20% EtOAc in pentane.
Yield: 70 mg (36%)
LC-MS (Method 2): Rt 2.68 min, m/z 538 [MH^+]

Intermediate 13



Intermediate 11 (150 mg, 0.27 mmol) was dissolved in acetonitrile (10 ml) and imidazole (111 mg, 1.636 mmol) and K_2CO_3 (226 mg, 1.636 mmol) were added. The reaction was heated at 70ºC for 7 h and then, after allowing to cool, the solvent was evaporated. The residue was dissolved in EtOAc (100 ml) and the solution was washed with water (50 ml) and brine (40 ml), dried (Na_2SO_4) and evaporated. The product was purified by chromatography on an Isolute Si(II) cartridge (20 g) eluting with 0-20% MeOH in EtOAc.
LC-MS (Method 2): Rt 2.68 min, m/z 538 [MH^+]

Intermediate 14

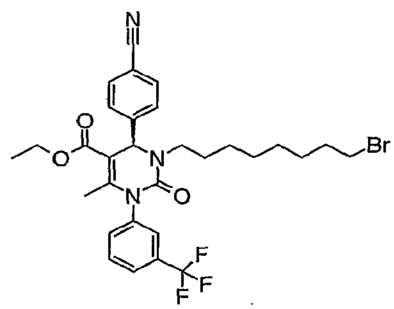


Intermediate 14 was prepared from Intermediate 1 and 1,8-dibromooctane by a similar procedure to that used in the synthesis of Intermediate 10.

Yield: 45%

LC-MS (Method 3): Rt 470/4.79 min, m/z 620/622 $[MH]^+$

Intermediate 15

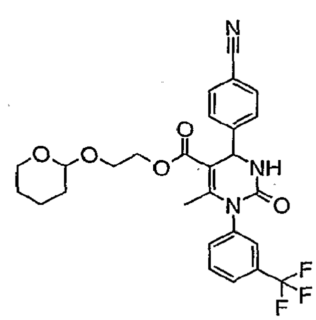


Intermediate 15 was prepared in a similar manner from Intermediate 5 and purified on an Isolute Si II cartridge eluting with 0-25% EtOAc in cyclohexane.

Yield: 57%

LC-MS (Method 2): Rt 4.89 min, m/z 620/622 $[MH^+]$

Intermediate 16



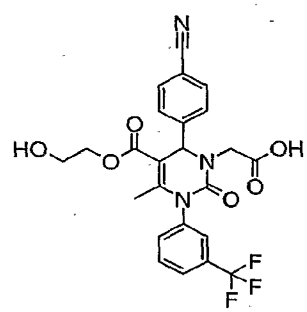
Intermediate 3 (7.0 g, 15.4 mmol) was dissolved in DCM (75 ml). EDC (5.02 g, 26.2

mmol) and DMAP (2.34 g, 19.2 mmol) were added and the solution was stirred for 5 min before the addition of 2-(tetrahydro-2H-pyran-2-yloxy)ethanol (3.19 g, 21.9 mmol). The mixture was allowed to stand at RT for 24 h and then washed with 1M aqueous Na_2CO_3 and brine, dried ($MgSO_4$) and evaporated. The resulting oil was chromatographed on silica gel using 0-10% EtOAc in DCM as eluent, giving Intermediate 16 as a pale yellow viscous oil.

Yield: 7.49 g (81%)

LC-MS (Method 2): Rt 3.82 min, m/z 530 [MH^+]

Intermediate 17

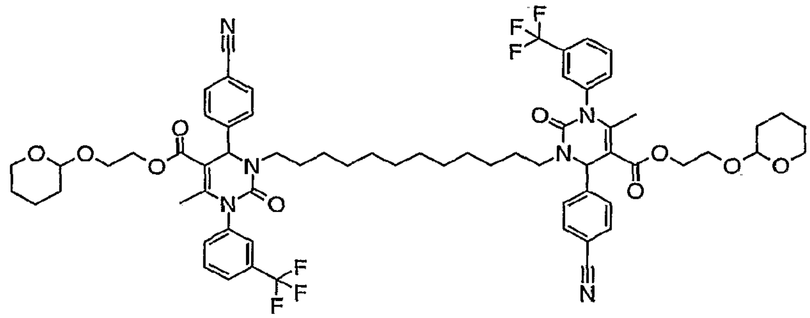


Intermediate 16 (2.00 g, 3.78 mmol) was dissolved in DMF (50 ml) and the solution was cooled to 0ºC under nitrogen. Sodium hydride (60% dispersion in mineral oil) (378 mg, 9.45 mmol) was added portion wise and the reaction was stirred for 15 min. The temperature was reduced to –10ºC and bromoacetic acid was added (526 mg, 3.78 mmol). The reaction was stirred whilst being allowed to warm to RT over 2 h, before being quenched by the addition of sat. aqueous NH_4Cl. The product was extracted into EtOAc (3×) and the combined organic extracts were washed with water then brine and finally dried (Na_2SO_4). Evaporation gave a brown oil which was loaded onto an Isolute NH_2 cartridge (25 g). The cartridge was flushed with acetonitrile and then the product was eluted with 2% conc. HCl in acetonitrile. The solvent was reduced and the residue was re-dissolved in MeOH (100 ml). Conc. HCl (3 ml) was added and after 30 min the solvent was reduced. The residue was treated with 1M NaOH (30 ml) in MeOH (100 ml) and the solution was acidified by the addition of 1M HCl. The product was extracted into EtOAc (3×) and the combined organics were washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified using HPLC system 2 and Intermediate 17 was obtained as a white solid.

Yield: 450 mg (24%)

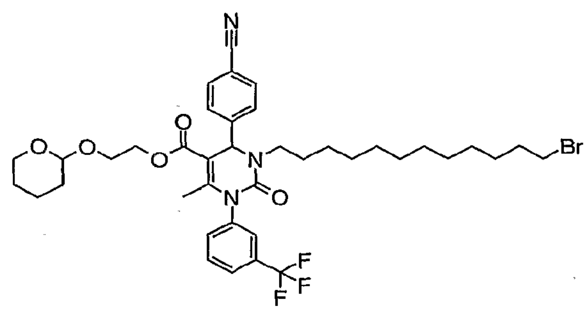
LC-MS (Method 2): Rt 3.16 min, m/z 504 $[MH]^+$

Intermediate 18



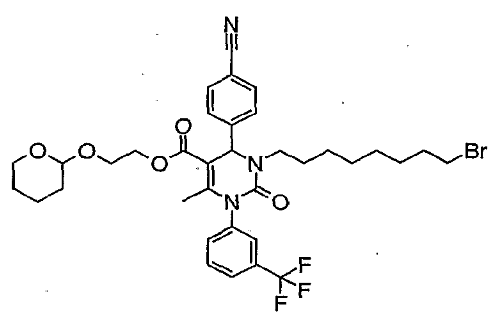
Intermediate 18 was prepared from Intermediate 16 and 0.5 equivalents of 1,12-dibromododecane by a similar procedure to that used in the synthesis of Intermediate 10.
LC-MS (Method 2): Rt 5.56 min, m/z 1057 $[MH-2xTHP]^+$

Intermediate 19



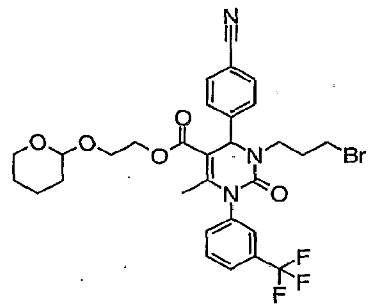
Intermediate 19 was obtained as a by-product in the synthesis of Intermediate 18.
LC-MS (Method 2): Rt 5.43 min.

Intermediate 20



Intermediate 20 was prepared from Intermediate 16 and 1,8-dibromooctane by a similar procedure to that used in the synthesis of Intermediate 10. The product was purified on an Isolute Si II cartridge eluting with 20% EtOAc in cyclohexane, then by HPLC system 4.
Yield: 17%
LC-MS (Method 2): Rt 4.92 min, m/z 720/722 $[MH^+]$

Intermediate 21

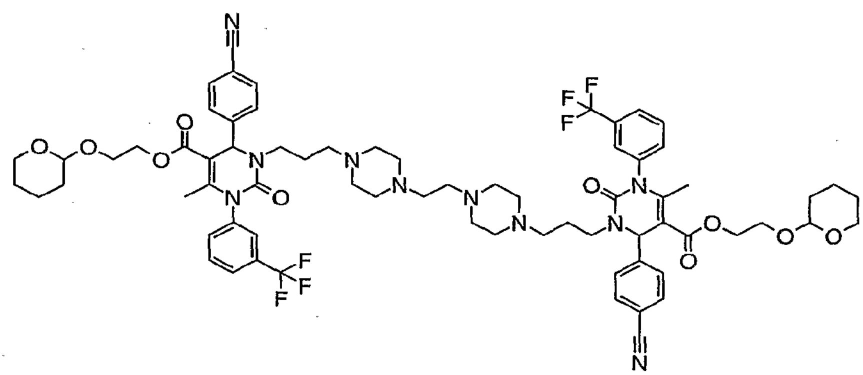


Intermediate 21 was prepared from Intermediate 16 and 1,3-dibromopropane by a similar procedure to that used in the synthesis of Intermediate 10. The product was purified on an Isolute Si II cartridge eluting with 20% EtOAc in cyclohexane.

Yield: 65%

LC-MS (Method 2): Rt 4.40 min, m/z 650 [MH^+]

Intermediate 22

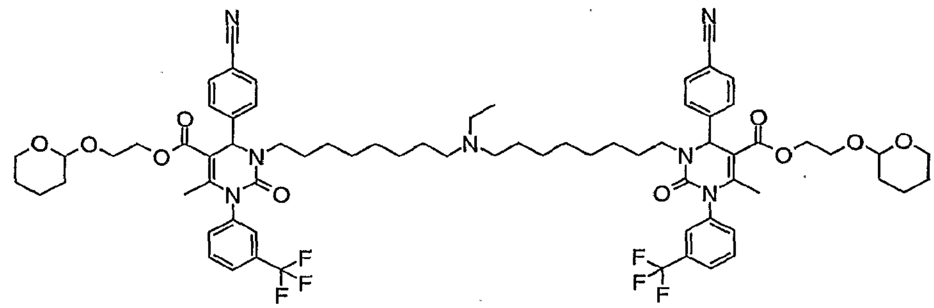


Intermediate 8 (129 mg, 0.32 mmol) was dissolved in DCM (1 ml) and treated with TFA (1 ml) for 30 min. The volatiles were evaporated and the residue was dissolved in acetonitrile (15 ml). $NaHCO_3$ (485 mg, 5.77 mmol) and Intermediate 21 (420 mg, 0.65 mmol) were added and the reaction mixture was heated at 80ºC for 24 h. The solvent was evaporated and the residue treated with DCM and the mixture filtered. After removal of the solvent the product was purified on an Isolute Si II cartridge (2 g) eluting with 0-10% MeOH in DCM.

Yield: 15%

LC-MS (Method 2): Rt 2.84 min, m/z 1337 [MH^+]

Intermediate 23

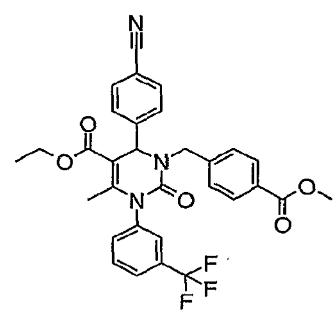


To a mixture of Intermediate 20 (110 mg, 0.153 mmol) and $NaHCO_3$ (40 mg, 0.478 mmol) in acetonitrile (5 ml) was added 2M ethylamine in THF (77 µl, 0.153 mmol). The reaction mixture was heated at 80°C for 24 h. The solvent was removed and the residue was purified on an Isolute Si II cartridge (2 g) eluting with 0-1% NH_3 in EtOAc as eluent.

Yield: 66 mg (65%)

LC-MS (Method 2): Rt 3.79 min, 1324 m/z [MH^+]

Intermediate 24

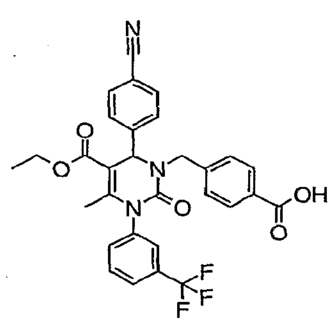


Intermediate 24 was prepared from Intermediate 1 and 1.2 equivalents of methyl 4-(bromomethyl)benzoate using a similar procedure to that used for the synthesis of Intermediate 10.

Yield: 80%

LC-MS (Method 2): Rt 4.29 min, m/z 578 $[MH]^+$

Intermediate 25

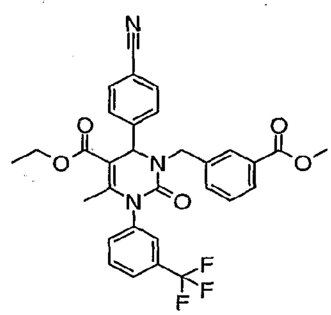


Intermediate 24 (320 mg, 0.55 mmol) was dissolved in THF (15 ml) and 1M NaOH (5 ml) was added. MeOH (1 ml) was added to aid dissolution and the reaction was stirred at RT for 2 h. The reaction mixture was acidified with 1M HCl and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried (Na_2SO_4) and evaporated. The product was purified using HPLC system 1 and obtained as a white foam.

Yield: 128 mg (41%)

LC-MS (Method 2): Rt 3.86 min, m/z 564 $[MH]^+$

Intermediate 26

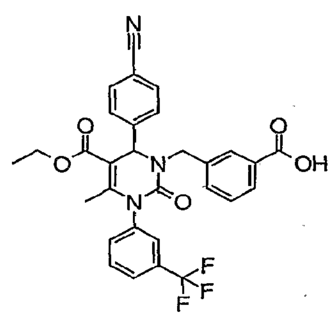


Intermediate 26 was prepared from Intermediate 1 and 1.2 equivalents of methyl 3-(bromomethyl)benzoate using a similar procedure to that used for the synthesis of Intermediate 10.

Yield: 83%

LC-MS (Method 2): Rt 4.28 min, m/z 578 $[MH]^+$

Intermediate 27

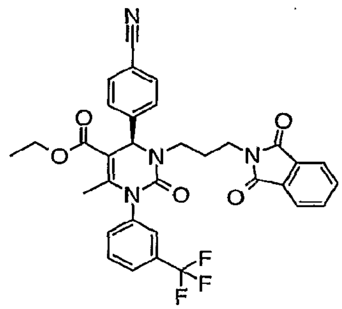


Intermediate 27 was prepared by a similar method to Intermediate 25 from Intermediate 26.

Yield: 29%

LC-MS (Method 2): Rt 3.92 min, m/z 564 $[MH]^+$

Intermediate 28

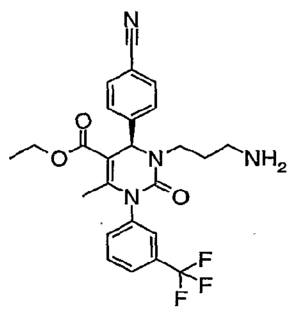


Intermediate 28 was prepared from Intermediate 5 and 1 equivalent of *N*-(3-bromopropyl)phthalimide using a similar procedure to that used to prepare Intermediate 10.

Yield: 69%

LC-MS (Method 4): Rt 4.19 min, m/z 617 $[MH]^+$

Intermediate 29

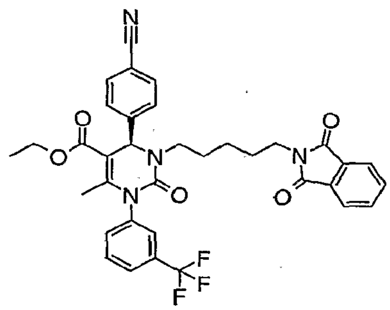


Intermediate 28 (882 mg, 1.43 mmol) was dissolved in IMS (45 ml) and 2M methylamine in THF (8.6 ml, 17.2 mmol) was added. The solution was stirred at 50ºC for 24 h and the volatiles were evaporated. The residue was dissolved in MeOH and loaded onto an Isolute SCX-2 cartridge. After flushing with MeOH, the product was eluted with 2M NH_3 in MeOH. Evaporation gave the desired product as a cream foam.

Yield: 422 mg (61%)

LC-MS (Method 3): Rt 2.46 min, m/z 487 $[MH]^+$

Intermediate 30

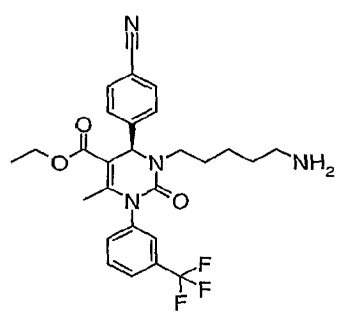


Intermediate 30 was prepared from Intermediate 5 and 1 equivalent of *N*-(5-bromopentyl)phthalimide using a similar procedure to that used to prepare Intermediate 10.

Yield: 39%

LC-MS (Method 3): Rt 4.28 min, m/z 645 $[MH]^+$

Intermediate 31

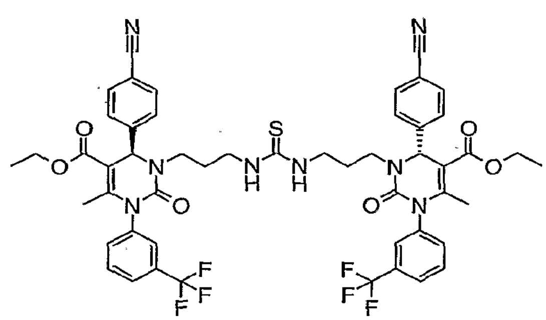


Intermediate 31 was prepared from Intermediate 30 a using a similar procedure to that used to prepare Intermediate 29.

Yield: 74%

LC-MS (Method 3): Rt 2.50 min, m/z 515 $[MH]^+$

Intermediate 32



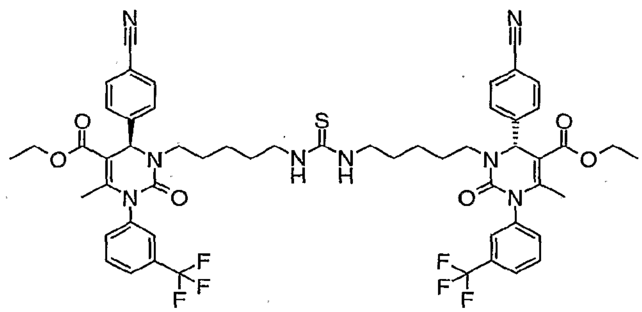
Intermediate 29 (372 mg, 0.77 mmol) was dissolved in DCM (15 ml) and 1,1'-thiocarbonyldipyridone (89 mg, 0.38 mmol) was added. The solution was allowed to stand at RT for 48 h. The solvent was removed and the residue was chromatographed

on an Isolute Si II cartridge (10 g) eluting with 50-80% EtOAc in pentane. The desired product was obtained as a colourless gum.

Yield: 263 mg (68%)

LC-MS (Method 2): Rt 4.66 min, m/z 1015 $[MH]^+$

Intermediate 33

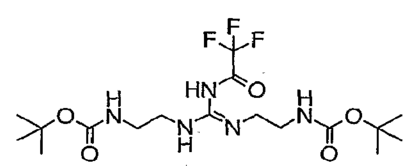


Intermediate 33 was prepared from Intermediate 31 by a method similar to that for the synthesis of Intermediate 32.

Yield: 63%

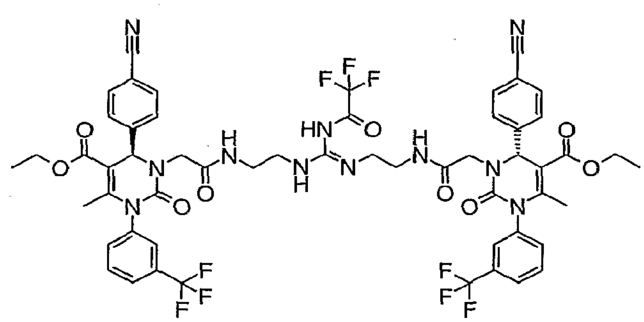
LC-MS (Method 3): Rt 4.63 min, m/z 1071 $[MH]^+$

Intermediate 34



Prepared according to Bartoli, S.; Jensen, K. B.; Kilburn, J. D. J. Org. Chem.: (2003) 68, 9416-9422.

Intermediate 35



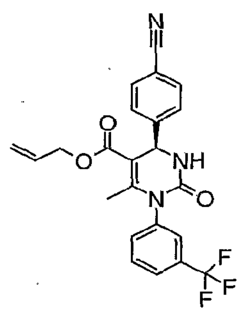
Intermediate 34 (181 mg, 0.41 mmol) was dissolved in 20% TFA in DCM (20 ml) and the solution was allowed to stand at RT for 3 h. Toluene was added, the solvents were

evaporated and the residue was re-dissolved in DMF (20 ml). Intermediate 7 (400 mg, 0.821 mmol) and DIPEA (1 ml) were added, followed by HATU (343 mg, 0.903 mmol). The solution was allowed to stand at RT until LC-MS indicated complete reaction (30 min) and the solvent was evaporated. The residue was dissolved in EtOAc (150 ml) and the organic solution was washed with sat. aqueous $NaHCO_3$ (100 ml), water (50 ml) and brine (50 ml). After drying over Na_2SO_4, the solvent was removed to give a residue which was purified by chromatography on an Isolute Si II cartridge (10 g) eluting with 60% EtOAc in pentane, 100% EtOAc then 10% MeOH in EtOAc.

Yield: 279 mg (57%)

LC-MS (Method 3): Rt 4.30 min, m/z 1180 $[MH]^+$

Intermediate 36

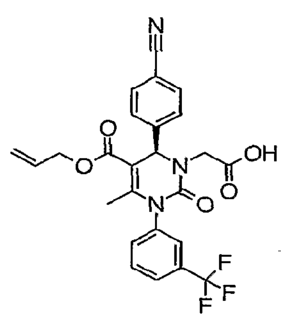


Intermediate 36 was prepared from Intermediate 4 and allyl alcohol using a similar method to that used for the preparation of Intermediate 5.

Yield: 57%

LC-MS (Method 3): Rt 3.65 min, m/z 442 $[MH^+]$

Intermediate 37

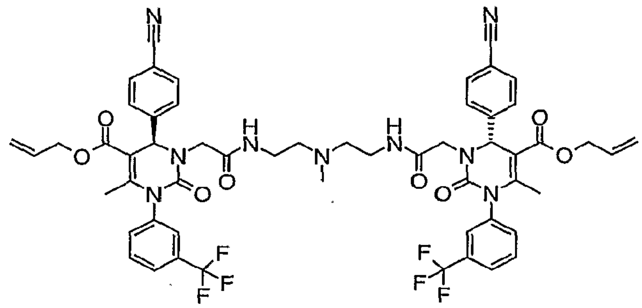


Intermediate 37 was prepared from Intermediate 36 by a similar method to that used for the preparation of Intermediate 7.

Yield: 84%

LC-MS (Method 2): Rt 3.77 min, m/z 500 $[MH^+]$

Intermediate 38

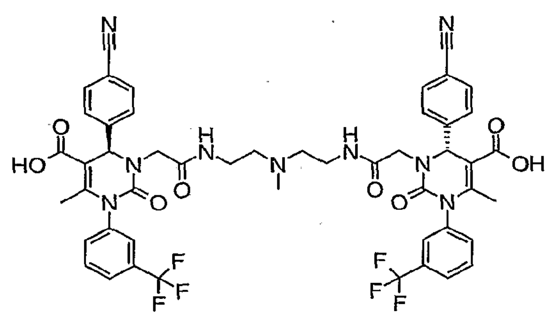


Intermediate 37 (1.14 g, 2.28 mmol), *N*-methyl-2,2′-diaminoethylamine (133 mg, 1.14 mmol) and DIPEA (2 ml) were dissolved in DMF (60 ml). HATU (953 mg, 2.51 mmol) was added and the solution was allowed to stand at RT until the reaction was complete by LC-MS (30 min). The DMF was evaporated and the residue was dissolved in EtOAc (200 ml). The organic solution was washed with aqueous $NaHCO_3$ (100 ml), water (50 ml) and brine (50 ml). After drying (Na_2SO_4), the solvent was removed to give a brown oil which was purified by chromatography on an Isolute Si II cartridge (25 g) eluting with 0-7% MeOH in EtOAc.

Yield: 800 mg (65%)

LC-MS (Method 3): Rt 3.05 min, m/z 1080 [MH^+]

Intermediate 39

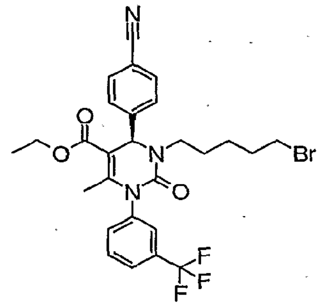


Intermediate 38 (800 mg, 0.74 mmol) was dissolved in THF (8 ml) and morpholine (648 mg, 7.45 mmol) and tetrakis(triphenylphosphine)palladium(0) (30 mg, 0.026 mmol) were added. The mixture was stirred at RT. After 3 h the mixture was diluted with EtOAc and the solution was washed with 10% aqueous HCl and brine. After drying (Na_2SO_4), the solvent was evaporated to give a solid which was triturated with diethyl ether. The di-acid was obtained as a yellow solid.

Yield: 742 mg (100%)

LC-MS (Method 3): Rt 2.70 min, m/z 1000 [MH^+]

Intermediate 40

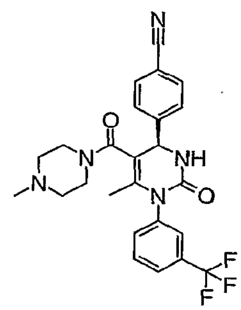


Intermediate 40 was prepared from Intermediate 5 and 1,5-dibromopentane by a similar method to that used for the synthesis of Intermediate 10.

Yield: 58%

LC-MS (Method 4): Rt 4.63 min, m/z 578/580 [MH$^+$]

Intermediate 41

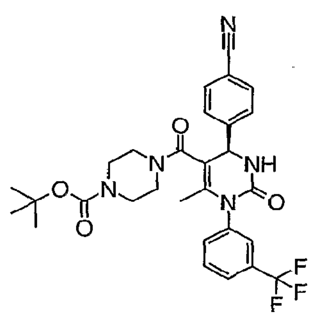


Intermediate 41 was prepared from Intermediate 4 and 1 equivalent of *N*-methylpiperazine by a similar method to that used in the synthesis of Intermediate 38. The product was purified by SCX chromatography.

Yield: 93%

LC-MS (Method 4): Rt 2.10 min, m/z 484 [MH$^+$]

Intermediate 42



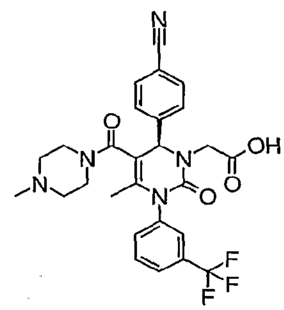
Intermediate 42 was prepared from Intermediate 4 and 1 equivalent of *N*-Boc piperazine

by a similar method to that used in the synthesis of Intermediate 38. The product was used without chromatographic purification.

Yield: 88%

LC-MS (Method 4): Rt 3.56 min, m/z 568 [M$^-$]

Intermediate 43

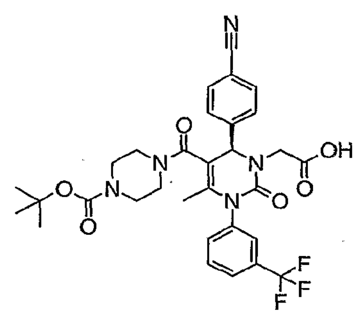


Intermediate 43 was prepared from Intermediate 41 by a similar method to that used for the preparation of Intermediate 7.

Yield: 29%

LC-MS (Method 4): Rt 2.22 min, m/z 542 [MH$^+$]

Intermediate 44

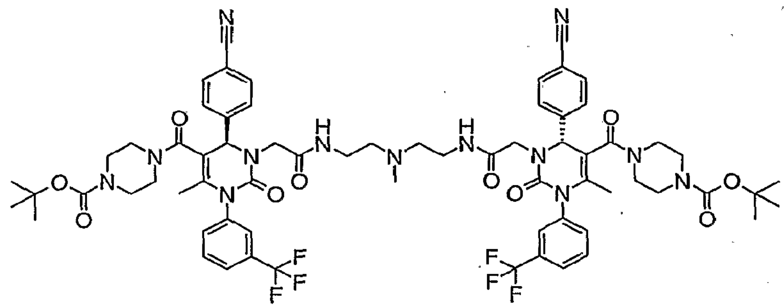


Intermediate 44 was prepared from Intermediate 42 by a similar method to that used for the preparation of Intermediate 7.

Yield: 93%

LC-MS (Method 4): Rt 3.61 min, m/z 628 [MH$^+$]

Intermediate 45

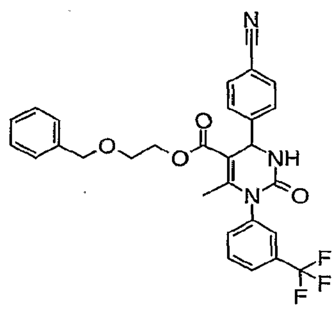


Intermediate 45 was prepared from Intermediate 44 and 0.5 equivalents of *N*-methyl-2,2′-diaminoethylamine by a similar method to that used in the synthesis of Intermediate 38. The product was purified by SCX chromatography.

Yield: 64%

LC-MS (Method 4): Rt 3.18 min, m/z 1336 [MH^+]

Intermediate 46

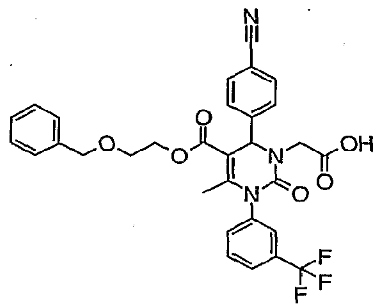


EDC (5.38 g, 28.1 mmol) and DMAP (2.51 g, 20.6 mmol) were added to a solution of Intermediate 3 (7.5 g, 18.7 mmol) in DCM (75 ml). 2-Benzyloxyethanol (3.32 ml, 23.4 mmol) was added to the mixture and the reaction was left to stir at room temperature overnight. The organics were washed with 1M aqueous Na_2CO_3 and brine. The combined aqueous layers were extracted with DCM, and the combined organic layers were washed with 1M aqueous HCl and brine. The organic layer was dried ($MgSO_4$) and the solvent removed to give a foam. Purification was achieved *via* column chromatography on silica gel eluting the product with 10% EtOAc in DCM. The main fraction (7.59 g) was a 1:1 mixture of the desired product and 2-benzyloxyethanol, but 0.26 g of pure product was also obtained. The 7.59 g batch was distilled to remove the alcohol (135ºC, ~15 mmHg). Purification was repeated to give 5.2 g of product contaminated with 10% of 2-benzyloxyethanol.

Yield: 55%

LC-MS (Method 3): Rt 3.82 min, m/z 536 [MH^+]

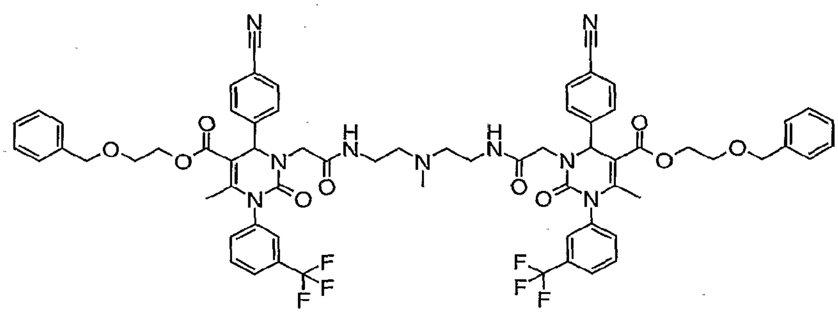
Intermediate 47



Intermediate 47 was prepared from Intermediate 46 using a method similar to that used to prepare Intermediate 7.

LC-MS (Method 3): Rt 3.75 min, m/z 594 [MH$^+$]

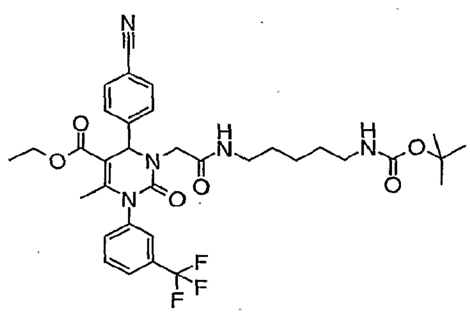
Intermediate 48



Intermediate 48 was prepared from Intermediate 47 and *N*-methyl-2,2′-diaminoethylamine by a method similar to the used in the synthesis of Intermediate 38.

LC-MS (Method 2): Rt 3.44 min, m/z 1268 [MH$^+$]

Intermediate 49

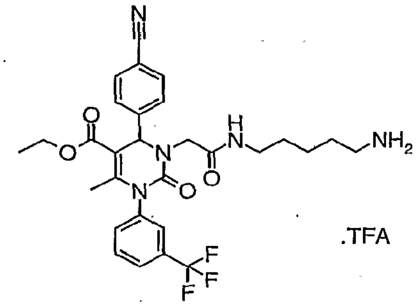


Intermediate 49 was prepared from Intermediate 6 and 1.1 equivalents of *N*-Boc-penta-1,5-diamine by a similar method to that used in the synthesis of Intermediate 38. The product was used without chromatographic purification.

Yield: 64%

LC-MS (Method 3): Rt 3.85 min, m/z 672 [MH$^+$]

Intermediate 50

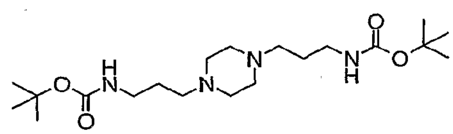


Intermediate 49 (100 mg, 0.149 mmol) was dissolved in DCM (10 ml) and treated with TFA (3 ml). After 2 h the volatiles were evaporated and the product was used without further purification.

Yield: 100%

LC-MS (Method 2): Rt 2.61 min, m/z 572 [MH^+]

Intermediate 51

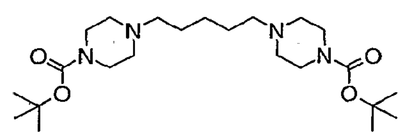


Piperazine (144 mg, 1.68 mmol), 3-(Boc-amino)propyl bromide (800 mg, 3.36 mmol) and $NaHCO_3$ (722 mg, 8.4 mmol) were stirred in acetonitrile (20 ml) at 90°C for 18 h. The solvent was removed and water (30 ml) and EtOAc (60 ml) were added then the layers separated. The organic layer was dried ($MgSO_4$) and evaporated.

Yield: 588 mg (88%)

LC-MS (Method 2): Rt 0.35/1.84 min, 401 m/z [MH^+]

Intermediate 52

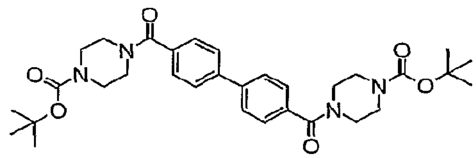


N-Boc piperazine (2.0 g, 10.75 mmol), glutaraldehyde (50% aqueous solution, 1.08 ml, 5.37 mmol) and sodium triacetoxyborohydride (3.68 g, 17.2 mmol) in DCE (30 ml) were stirred at RT under nitrogen for 3 h. The reaction mixture was quenched using sat. aqueous $NaHCO_3$, extracted with EtOAc, dried ($MgSO_4$) and evaporated to give a colourless oil which solidified on standing.

Yield: 2.00 g (42%)

LC-MS (Method 3): Rt 0.26/1.5 min, 441 m/z [MH^+]

Intermediate 53

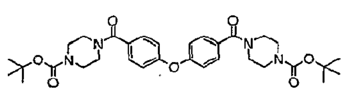
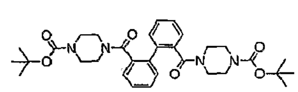
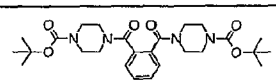
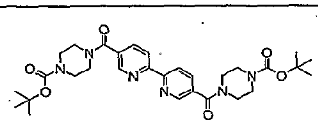
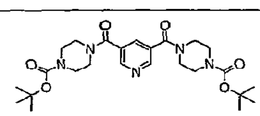


4,4'-Biphenyldicarboxylic acid (2.0 g, 8.3 mmol), *N*-Boc-piperazine (3.38 g, 18.2 mmol), HATU (6.55 g, 18.2 mmol) and DIPEA (9.4 ml, 55 mmol) were suspended in DMF (30 ml) and stirred at RT for 30 min. The mixture was divided into three portions and each was irradiated in the microwave at 100°C for 5 min before the samples were re-combined, diluted with diethyl ether and filtered. The solid was washed with diethyl ether and suction dried to an off-white powder.

Yield: 3.04 g (63%)

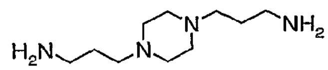
LC-MS (Method 2): Rt 3.79 min, 579 m/z [MH^+]

The following compounds were prepared from a di-acid and *N*-Boc-piperazine by a procedure similar to that used for the synthesis of Intermediate 53:

Compound	Structure	Di-acid	Reaction time	Yield (%)	Rt (min) (Method 2)	Mass [MH]$^+$
Intermediate 54		4,4'-Oxybisbenzoic	30 min	49	*See NMR data	
Intermediate 55		Diphenic	10 min (100ºC µwave)	83	3.88	579
Intermediate 56		Phthalic	24 h	83	3.40	525 [M+Na$^+$]
Intermediate 57		2,2'-Bipyridine-5,5'-dicarboxylic	17 h	74	3.19	581
Intermediate 58		3,5-Pyridinedicarboxylic	17 h	96	2.90	504

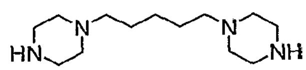
*^{1}H-NMR (400 MHz, MeOD): δ = 7.47 (1H, d); 7.11 (1H, d); 3.32-3.60 (16h, br s); 1.41 (18H, s)

Intermediate 59



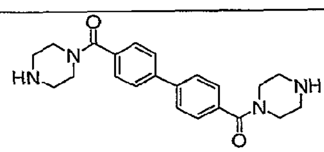
Intermediate 51 (550 mg, 1.375 mmol) was stirred in 1.25 M HCl in MeOH (10 ml) at RT for 1h then evaporated.
Yield: 370 mg (99%)
LC-MS (Method 2): Rt 0.25 min, 201 m/z [MH$^+$]

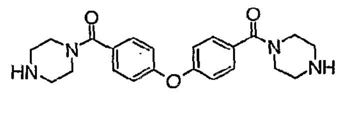
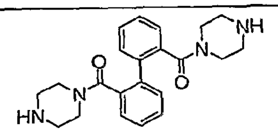
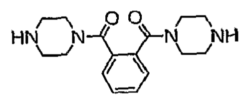
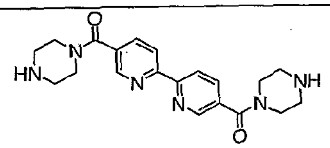
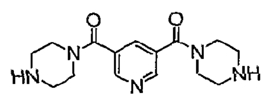
Intermediate 60



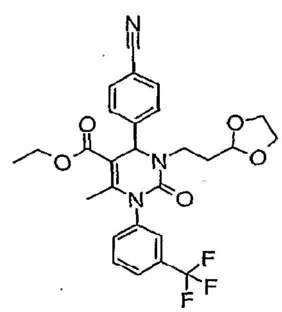
Intermediate 52 (1.0 g, 2.27 mmol) was dissolved in DCM (4 ml) and TFA (4 ml) was added. The mixture was stirred at RT for 30 min. The solvent was removed and the residue was taken up into 1:1 DCM/ MeOH and applied to an SCX-2 cartridge. After washing with 1:1 DCM/ MeOH then MeOH, the product was eluted with 2M NH_3 in MeOH. The solvent was removed.
Yield: 676 mg (100% (contains solvent))
LC-MS (Method 2): Rt 0.26 min, 241 m/z [MH$^+$]

The following compounds were prepared using a procedure similar to that used for the synthesis of Intermediate 60:

Compound No.	Structure	Starting materials	Reaction time (min)	Yield (%)	Rt (min) (Method 1)	Mass [MH]$^+$
Intermediate 61		Intermediate 53	30	100	0.33	379

Intermediate 62		Intermediate 54	30	70	0.35	395
Intermediate 63		Intermediate 55	30	100	0.33	379
Intermediate 64		Intermediate 56	30	91	0.28	303
Intermediate 65		Intermediate 57	30		0.23	381
Intermediate 66		Intermediate 58	30	100	0.19	304/607

Intermediate 67

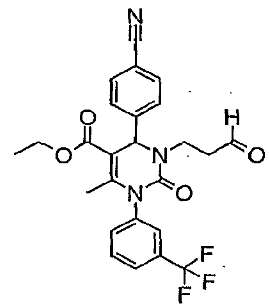


Intermediate 67 was prepared from Intermediate 1 and 6 equivalents of 2-(2-bromoethyl)-1,3-dioxolane using a method similar to that used for the preparation of Intermediate 10. After removal of the DMF, the product was purified directly on neutral alumina using 0-5% EtOH in DCM.

Yield: 77%

LC-MS (Method 2): Rt 4.03 min, 530 m/z [MH^+]

Intermediate 68

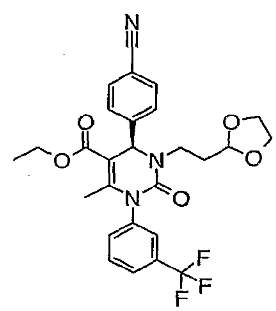


A solution of Intermediate 67 (3.4 g, 6.43 mmol) was dissolved in THF (20 ml), 1 M HCl (50 ml) was added and the reaction was stirred at 80°C overnight. The solvent was reduced and DCM (50 ml) and water (30 ml) were added. The phases were separated before the organic layer was washed with brine and dried ($MgSO_4$). The crude material was purified by chromatography using 0-2% MeOH in DCM.

Yield: 2.38 g (76%)

LC-MS (Method 3): Rt 3.20/ 3.67 min, 486 m/z [MH^+]

Intermediate 69

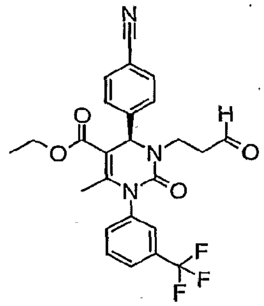


Intermediate 69 was prepared from Intermediate 5 by a similar procedure to that used for the synthesis of Intermediate 10. The product was purified by addition of cyclohexane, decanting off the liquid and then repeating.

Yield: 5.18 g (70%)

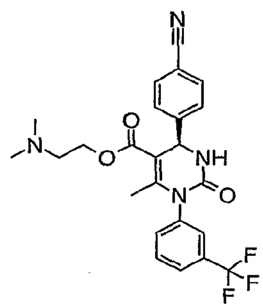
LC-MS (Method 3): Rt 3.78 min, 530 m/z [MH^+]

Intermediate 70



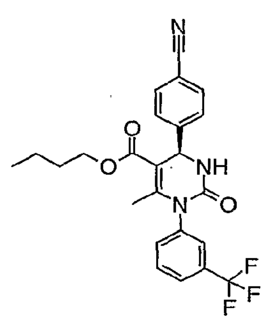
Intermediate 70 was prepared from Intermediate 69 by the method described for the synthesis of Intermediate 68.
Yield: 1.82 g (38%)
LC-MS (Method 3): Rt 3.67 min, 486 m/z [MH^+]

Intermediate 71



Intermediate 71was prepared from Intermediate 4 and *N,N*-dimethylethanolamine by a procedure similar to that used for the synthesis of Intermediate 5. The crude material was purified by column chromatography using 0% then 10% MeOH in DCM. The material obtained was further purified by loading onto a PE-AX cartridge and washing with acetonitrile then evaporation of the solvent.
Yield: 311 mg (44%)
LC-MS (Method 2): Rt 2.38/2.47 min, 473 m/z [MH^+]

Intermediate 72



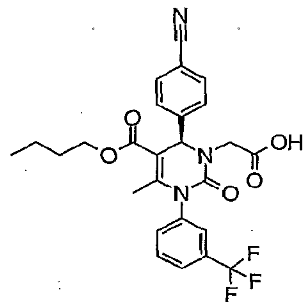
Intermediate 72 was prepared from Intermediate 4 and n-butanol by a procedure similar

to that used for the synthesis of Intermediate 5.

Yield: 54%

LC-MS (Method 2): Rt 4.14 min, 458/915 m/z [MH^+]

Intermediate 73

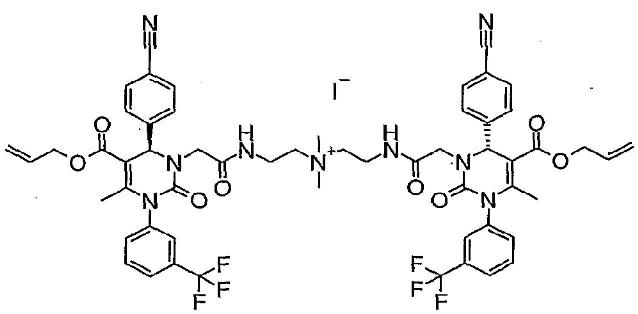


Intermediate 73 was prepared from Intermediate 72 by a procedure similar to that used for the synthesis of Intermediate 7, but the product was used without purification on an NH_2 cartridge.

Yield: 49%

LC-MS (Method 3): Rt 3.77 min, 514 m/z [$M-H^+$]

Intermediate 74

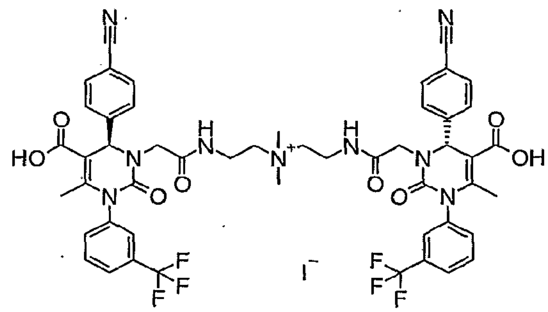


Intermediate 38 (450 mg, 0.417 mmol) was dissolved in acetonitrile (10 ml) and $NaHCO_3$ (105 mg, 1.25 mmol) and iodomethane (1 ml) were added. The solution was stirred at RT for 3.7 h, filtered and evaporated.

Yield: 496 mg (97%)

LC-MS (Method 4): Rt 3.28 min, 1094 m/z [MH^+]

Intermediate 75

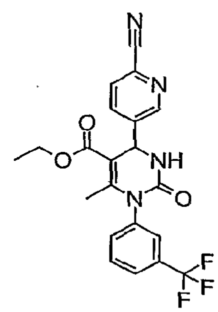


Intermediate 75 was prepared from Intermediate 74 according to method described for Intermediate 39.

Yield: 98%

LC-MS (Method 4): Rt 2.80min, 1015 m/z [MH^+]

Intermediate 76

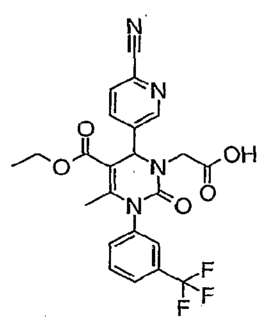


Intermediate 76 was prepared by a procedure analogous to that used for the synthesis of Intermediate 2. The product was purified by column chromatography using 0-10% EtOAc in DCM.

Yield: 34%

LC-MS (Method 1): Rt 10.62 min, 430 m/z [MH^+]

Intermediate 77

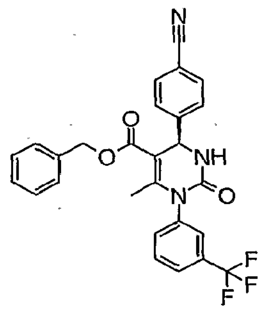


Intermediate 77 was prepared from Intermediate 76 by a procedure similar to that used for the synthesis of Intermediate 7, but the product was used without purification.

Yield: 55%

LC-MS (Method 3): Rt 3.27 min, 489 m/z [MH^+]

Intermediate 78

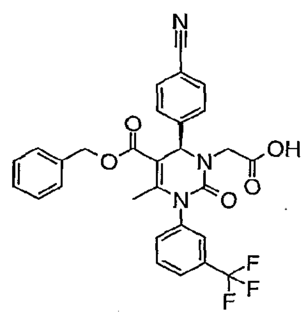


Intermediate 4 (2.00g, 4.98 mmol), benzyl bromide (600 µl, 4.98 mmol) and K_2CO_3 (1.38 g, 1.00 mmol) in DMF (50 ml) were stirred at RT for 6.5 h. Sat. aqueous NH_4Cl solution (50 ml) was added and the organic layer was separated. Evaporation gave a straw coloured glass.

Yield: 2.43 g (99%)

LC-MS (Method 2): Rt 4.09 min, 492 m/z [MH^+]

Intermediate 79

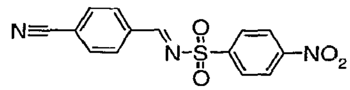


Intermediate 79 was prepared from Intermediate 78 using a similar procedure to that used for Intermediate 7.

Yield: 29%

LC-MS (Method 2): Rt 3.99 min, 550 m/z [MH^+]

Intermediate 80

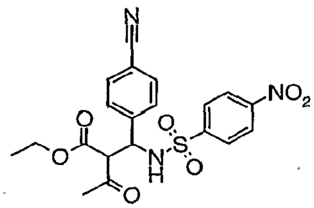


4-Cyanobenzaldehyde (3.24 g, 24.75 mmol) and 4-nitrophenylsulphonamide (5.00 g, 24.75 mmol) were stirred in DCM (100 ml) whilst BF_3 etherate was added. The reaction was stirred for a further 4 h after which time the solid was filtered off and washed with

diethyl ether.

Yield: 4.40 g, 56%

Intermediate 81

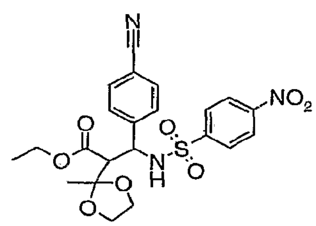


Intermediate 80 (2.90 g, 9.21 mmol) was suspended in THF (60 ml) and triethylamine (3.8 ml, 27.6 mmol) was added, followed by ethyl acetoacetate (1.32 g, 10.13 mmol). The reaction was stirred at RT for 1.5 h. The mixture was filtered and the solvent was evaporated. The residue was dissolved in a minimum amount of EtOAc and diethyl ether (150 ml) was added with rapid stirring. After 15 min, the white solid was filtered off, washed with diethyl ether and dried.

Yield: 2.88 g (70%)

LC-MS (Method 2): Rt 3.47 min, m/z 444 [$M-H^+$]

Intermediate 82

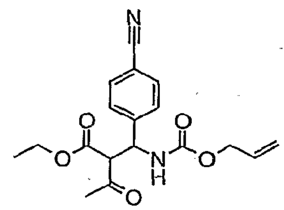


Intermediate 81 (1.189 g, 2.67 mmol), ethylene glycol (446 μl, 8.01 mmol) and 4-toluenesulphonic acid (51 mg, 0.27 mmol) in toluene were heated at reflux under Dean-Stark conditions (a slight vacuum was applied to the apparatus). After 6 h the reaction mixture was allowed to cool and the toluene was removed by evaporation. The residue was partitioned between DCM (200 ml) and sat. aqueous sodium hydrogen carbonate (150 ml). The organic layer was separated, washed with brine (50 ml), dried (Na_2SO_4) and evaporated. The resulting cream solid was triturated with diethyl ether and the product was obtained as a white solid.

Yield 665 mg (51%)

LC-MS (Method 2): Rt 3.49 and 3.57 min, m/z 488 [M-H] diasteromeric mixture.

Intermediate 83

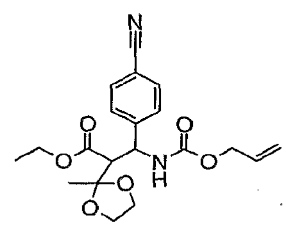


To a solution of allyl carbamate (100 mg, 1.0 mmol) and 4-cyanobenzaldehyde (131 mg, 1.0 mmol) in dry DCM (3 ml) at -78°C was added BF_3 etherate (246 μl, 2.0 mmol) and the mixture warmed to RT and stirred for 20 min. This solution was added dropwise to a stirring suspension of (-)-cinchonidine (29 mg, 0.1 mmol) and ethyl acetoacetate (127 μl, 1.0 mmol) in dry DCM (3 ml) at -35°C, which had previously been stirred together for 5 mins. The reaction mixture was stirred at -35°C for 17h then the reaction mixture was filtered through a pad of silica, washing with EtOAc, then evaporated. The crude material was purified by chromatography using 10-25% EtOAc in cyclohexane to give Intermediate 83.

Yield: 98 mg (28%)

LC-MS (Method 3): Rt 3.12 min, 367 m/z [M+Na$^+$]

Intermediate 84

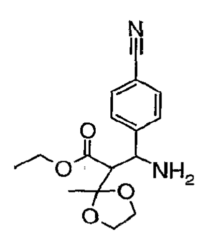


Intermediate 84 was prepared from Intermediate 83 by a similar method to that used in the synthesis of Intermediate 82. The product was triturated with ether to afford Intermediate 84, which was used without further purification.

Yield: 845 mg (76%)

LC-MS (Method 3): Rt 3.24 min, 411 m/z [MNa$^+$]

Intermediate 85



Method A: From Intermediate 82

Octanethiol (597 mg, 4.090 mmol) was dissolved in DMF (10 ml) and sodium hydride (60% dispersion in mineral oil) was added. The reaction was stirred at RT and after 15 min, Intermediate 82 (500 mg, 1.022 mmol) was added. The solution was stirred for 3 h and then treated with 2 equivalents of acetic acid. The mixture was loaded directly onto an Isolute SCX-2 cartridge (20 g). After flushing with MeOH the product was eluted with 2M NH_3 in MeOH. The fractions containing UV-active material were combined and evaporated.

Yield: 343 mg (100%)

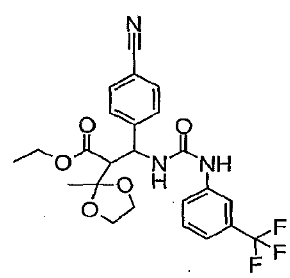
Method B: From Intermediate 84

A flask was charged with $Pd(PPh_3)_4$ (8 mg, 0.0072 mmol) and 1,3-dimethylbarbituric acid (56 mg, 0.361 mmol) and Intermediate 84 (140 mg, 0.361 mmol) was added as a solution in dry DCM (2 ml). The reaction mixture was stirred under argon for 1 h and then filtered through a pad of silica and evaporated. The crude material was applied to a MeOH preconditioned SCX-2 cartridge, washed with MeOH and the product eluted using 2N NH_3 in MeOH. Evaporation afforded Intermediate 85.

Yield: 57 mg (52%)

LC-MS (Method 2): Rt 0.34 min, 305 m/z [MH^+]

Intermediate 86

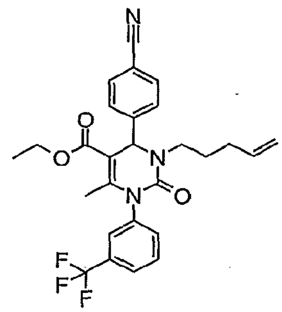


To a solution of Intermediate 85 (303 mg, 0.81 mmol) in DCM (6 ml) was added 3-(trifluoromethyl)phenyl isocyanate (111 µl, 0.806 mmol) and the reaction mixture was stirred for 1h. The solvent was evaporated to afford the product, which was used without further purification.

Yield: 430 mg (95%)

LC-MS (Method 2): Rt 4.27min, 560 [MH+]; 582 m/z [MNa+]

Intermediate 87



Method A

Intermediate 1 (195 mg, 0.349 mmol) was dissolved in IMS (2 ml) and conc. aqueous HCl (100 µl) was added. The solution was heated in the microwave at 120ºC for 10 min. the solvent was evaporated and the residue was partitioned between DCM (10 ml) and sat. aqueous sodium hydrogen carbonate (10 ml). The organic phase was separated and evaporated. The residue was chromatographed on an Isolute SCX-2 cartridge (10 g) eluting with 0-30% ethyl actate in pentane. The product was obtained as a colourless gum.

Yield: 43 mg (25%)

LC-MS (Method 3): Rt 4.32 min, m/z 498 [MH$^+$]

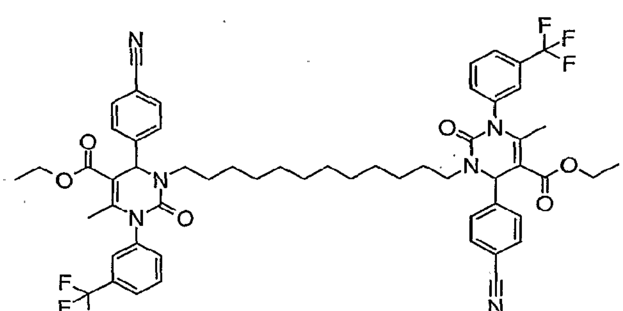
Method B

From Intermediate 1 and 2 equivalents of 5-bromopent-1-ene using a similar procedure to that used in the synthesis of Intermediate 10.

Yield: 65%

LC-MS (Method 3): Rt 4.32 min, m/z 498 [MH$^+$]

Example 1



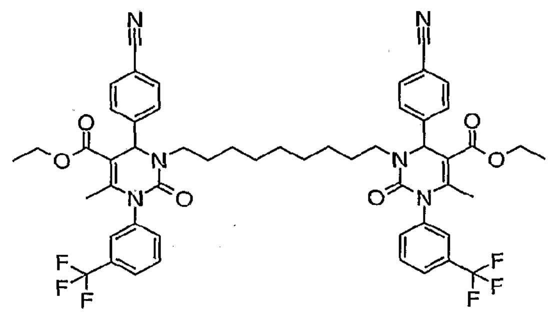
Example 1 was prepared from Intermediate 1 and 0.5 equivalents of 1,12-dibromododecane using a method similar to that used for the preparation of Intermediate

10. The product was purified by HPLC system 1.

Yield: 104 mg (30%)

LC-MS (Method 1): Rt 17.53, m/z 1025.12 $[MH]^+$

Example 2

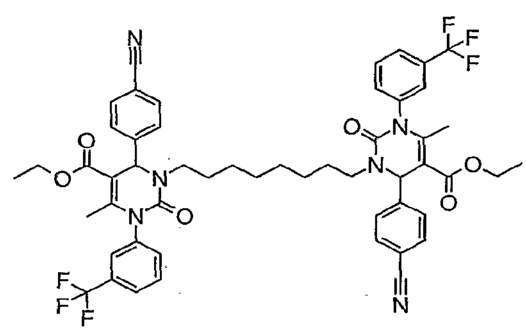


Example 2 was prepared from Intermediate 1 and 0.5 equivalents of 1,9-dibromononane using a method similar to that used for the preparation of Intermediate 10. The product was purified by HPLC system 1.

Yield: 22%

LC-MS (Method 1): Rt 16.50 min, m/z 983.09 $[MH^+]$

Example 3

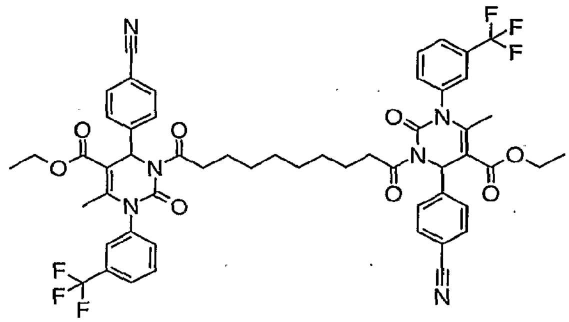


Example 3 was prepared from Intermediate 1 and 0.5 equivalents of 1,8-dibromooctane using a method similar to that used for the preparation of Intermediate 10. The product was purified by HPLC system 1.

Yield: 6%

LC-MS (Method 1): Rt 16.24 min, m/z 968.92 $[MH^+]$

Example 4

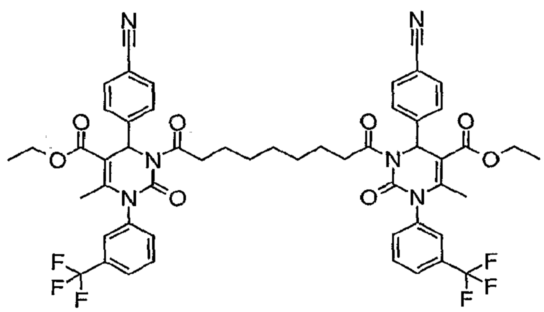


Example 4 was prepared from Intermediate 1 and 0.5 equivalents of sebacoyl chloride using a method similar to that used for the preparation of Intermediate 10, but using THF as the reaction solvent. The product was purified by MPLC on silica gel eluting with 25% EtOAc in pentane.

Yield: 94 mg (32%)

LC-MS (Method 1): Rt 16.42 min, m/z 1025.10 [MH$^+$]

Example 5

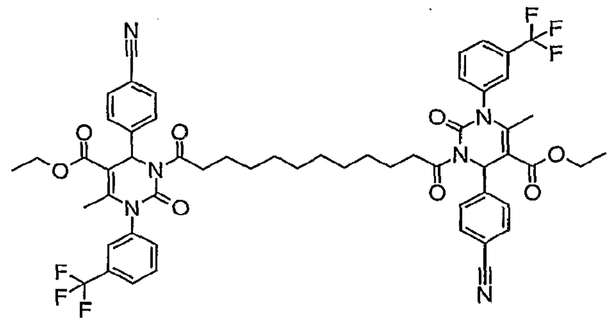


Example 5 was prepared from Intermediate 1 and 0.5 equivalents of azelaoyl chloride using a method similar to that used for the preparation of Intermediate 10 but using THF as the reaction solvent. Purification was carried out on an Isolute Si II column eluting with 0-15% EtOAc in cyclohexane, followed by HPLC (System I).

Yield: 44%

LC-MS (Method 1): Rt 16.10 min, m/z 1010.91 [MH$^+$]

Example 6

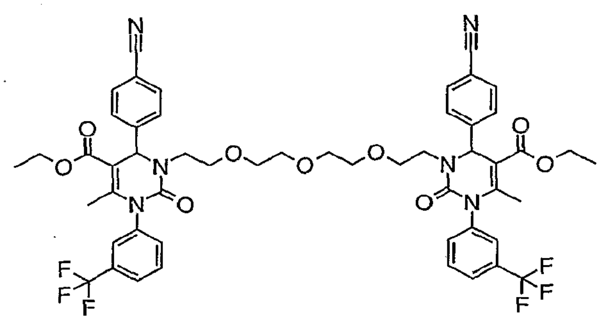


Example 6 was prepared from Intermediate 1 and 0.5 equivalents of dodecanedioyl dichloride using a method similar to that used for the preparation of Intermediate 10, but using THF as the reaction solvent. This compound was purified by HPLC system 1.

Yield: 50%

LC-MS (Method 1): Rt 16.93 min, m/z 1053.06 [MH$^+$]

Example 7

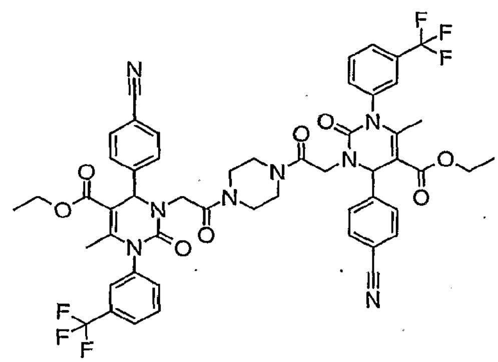


Example 7 was prepared from Intermediate 1 and 0.5 equivalents of Intermediate 9 using a method similar to that used for the preparation of Intermediate 10. This compound was purified twice by HPLC System 1 and then on an Isolute Si II cartridge (10 g) eluting with 0-30% EtOAc in pentane.

Yield: 30 mg (4%)

LC-MS (Method 1): Rt 15.15 min, m/z 1017.08 [MH$^+$]

Example 8

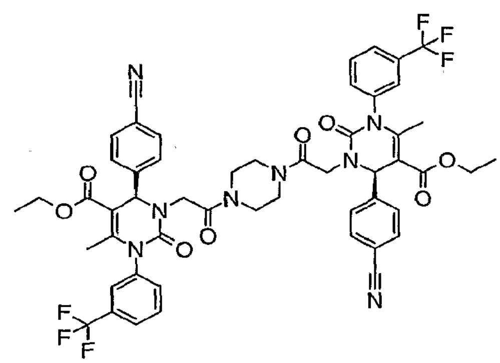


Example 8 was prepared from Intermediate 6 and 0.5 equivalents of piperazine using a similar procedure to that used for the synthesis of Intermediate 38. Purification was achieved using HPLC system 1.

Yield: 19%

LC-MS (Method 1): Rt 13.64 min, m/z 1025.10 [MH$^+$]

Example 9

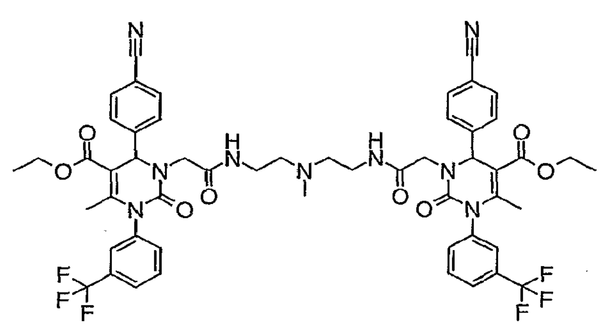


Example 9 was prepared in a similar manner from Intermediate 7 and purified using HPLC system 3.

Yield: 48%

LC-MS (Method 1): Rt 13.40 min, m/z 1025.22 [MH$^+$]

Example 10



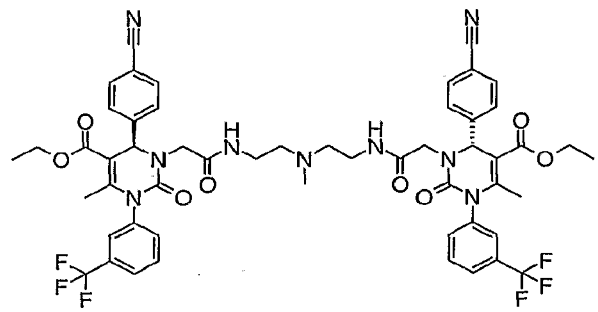
Example 10 was prepared from Intermediate 6 and 0.5 equivalents of *N*-methyl-2,2′-

diaminoethylamine using a similar procedure to that used for the synthesis of Intermediate 38. Purification was achieved using HPLC system 1.

Yield: 11%

LC-MS (Method 1): Rt 10.44 min, m/z 1056.17 [MH$^+$]

Example 11

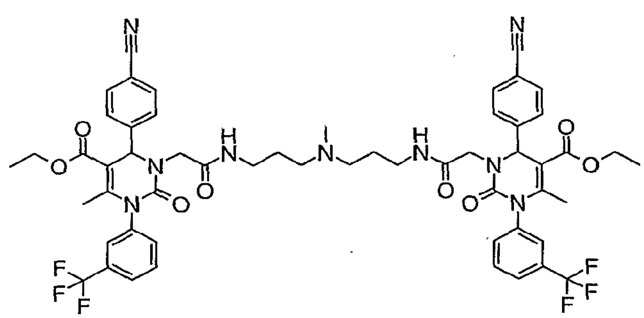


Example 11 was prepared in a similar manner from Intermediate 7 and purified using HPLC system 3.

Yield: 50%

LC-MS (Method 1): Rt 10.10 min, m/z 1056.21 [MH$^+$]

Example 12

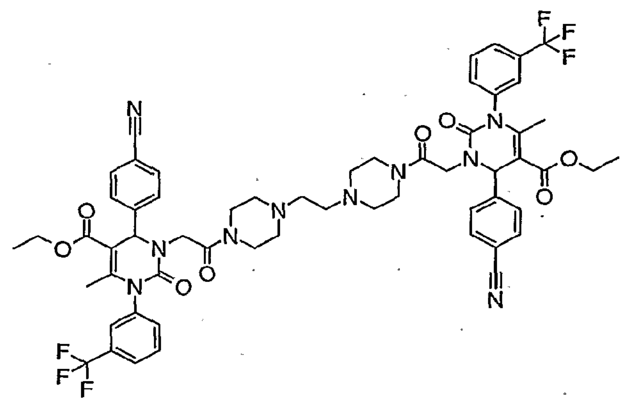


Example 12 was prepared from Intermediate 6 and 0.5 equivalents of 3,3′-diamino-*N*-methyldipropylamine using a similar procedure to that used for the synthesis of Intermediate 38. Purification was achieved using HPLC system 1.

Yield: 6%

LC-MS (Method 1): Rt 10.38 min, m/z 1084.30 [MH$^+$]

Example 13

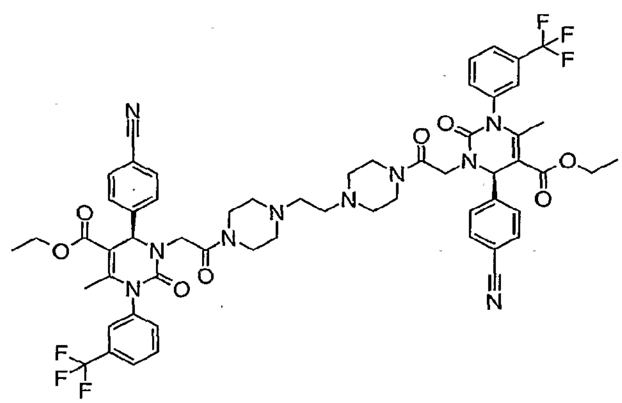


Intermediate 8 (61 mg, 0.154 mmol) was dissolved in 25% TFA in DCM (15 ml) and the solution was allowed to stand at RT for 1.5 h. The solvents were evaporated and the residue was re-dissolved in DMF (5 ml). Intermediate 6 (150 mg, 0.308 mmol) and DIPEA (161 µl, 0.924 mmol) were added, followed by HATU (176 mg, 0.462 mmol). The solution was allowed to stand at RT for 17 h and the solvent was evaporated. The residue was dissolved in EtOAc (60 ml) and the organic solution was washed with aqueous $NaHCO_3$ (30 ml), water (30 ml) and brine (20 ml). After drying (Na_2SO_4), the solvent was removed to give a residue which was purified by HPLC System 1.

Yield: 21%

LC-MS (Method 1): Rt 10.35 min, m/z 1136.97 [MH^+]

Example 14

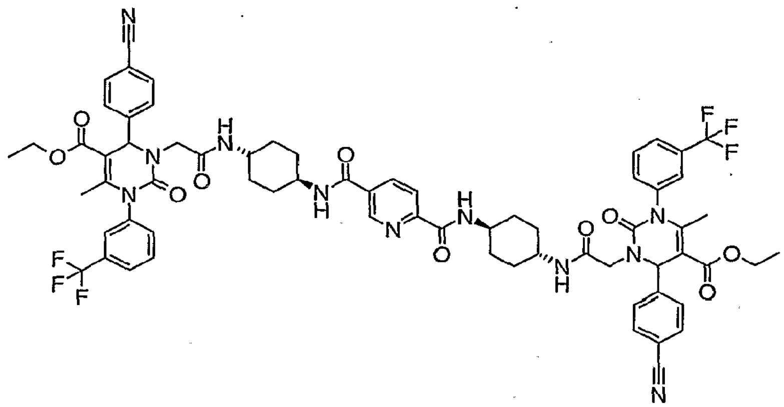


Example 14 was prepared in a similar manner from Intermediate 7 and purified using HPLC system 3.

Yield: 32%

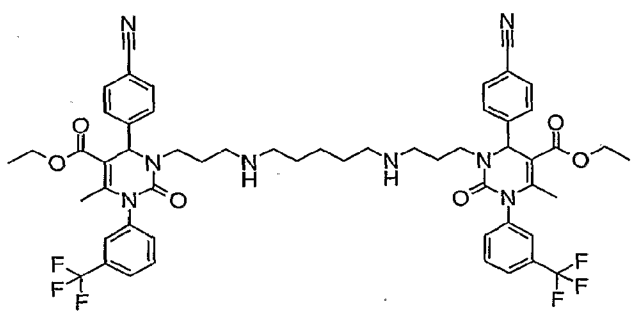
LC-MS (Method 1): Rt 10.14 min, m/z 1137.29 [MH^+]

Example 15



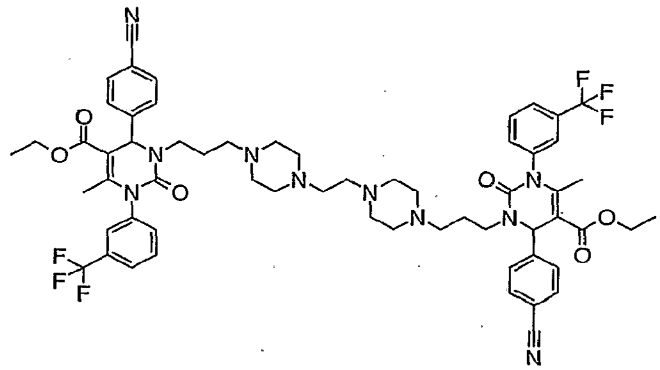
Example 15 was prepared by a similar procedure from Intermediate 6. The product was purified by HPLC system 1 and then HPLC system 5.
Yield: 5%
LC-MS (Method 1): Rt 13.42 min, m/z 1298.26 [MH$^+$]

Example 16



Intermediate 10 (98 mg, 0.178 mmol), 1,5-dibromopentane (8 mg, 0.080 mmol) and $NaHCO_3$ (60 mg, 0.712 mmol) in acetonitrile (5 ml) were heated at 80°C for 17 h. After cooling to RT the solvent was reduced and the mixture was purified by HPLC system 1.
Yield: 22 mg (22%)
LC-MS (Method 1): Rt 9.28 min, m/z 1041.13 [MH$^+$]

Example 17

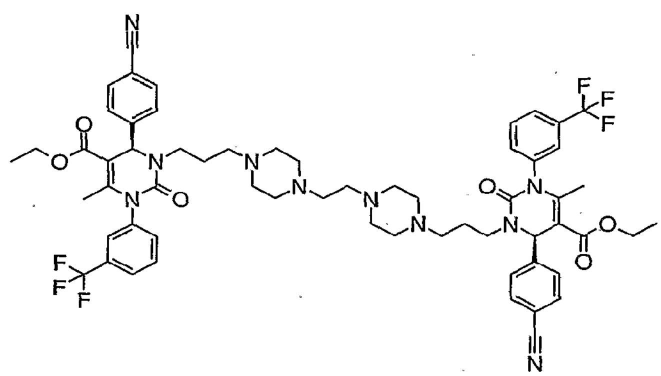


Intermediate 8 (72 mg, 0.181 mmol) was dissolved in 25% TFA in DCM (30 ml) and the solution was allowed to stand at RT for 1 h. The solvents were evaporated and the residue was re-dissolved in acetonitrile (10 ml). Intermediate 10 (200 mg, 0.364 mmol), and $NaHCO_3$ (276 mg, 3.286 mmol) were added and the mixture was heated at 80°C for 17 h. After cooling to RT and evaporation of the solvent, the residue was purified by HPLC system 1.

Yield: 65 mg (29%)

LC-MS (Method 1): Rt 8.91 min, m/z 1137.32 [MH^+]

Example 18

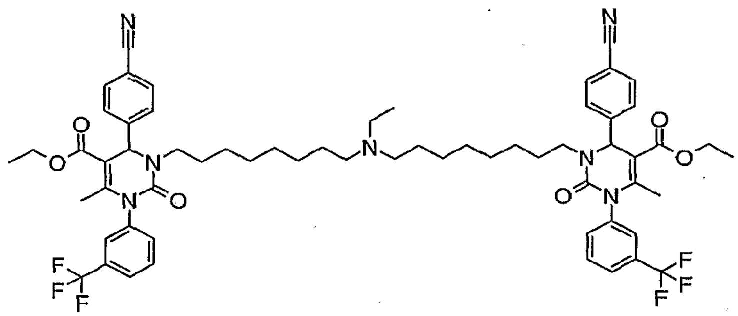


Example 18 was prepared in a similar manner from Intermediate 11 and purified using HPLC system 1.

Yield: 8%

LC-MS (Method 1): Rt 8.69 min, m/z 1137.44 [MH^+]

Example 19

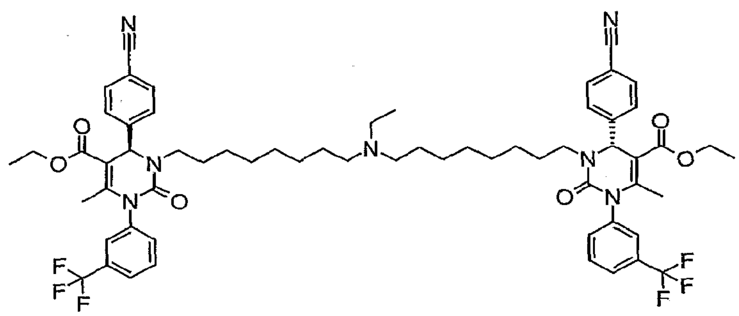


To a mixture of Intermediate 14 (33 mg, 0.0532 mmol) and $NaHCO_3$ (13 mg, 0.160 mmol) in acetonitrile (2 ml) was added 2M ethylamine in THF (53 µl, 0.106 mmol). The reaction mixture was heated at 80°C for 41 h. The solvent was removed and the residue was purified using HPLC system 1.

Yield: 14 mg (21%)

LC-MS (Method 1): Rt 12.89 min, 1124.30 m/z [MH^+]

Example 20

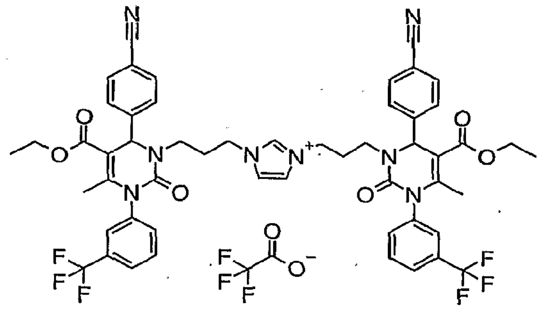


To a solution of Intermediate 15 (248 mg, 0.40 mmol) in acetonitrile (15 ml) was added $NaHCO_3$ (101 mg, 1.2 mmol) and 2M ethylamine in THF (400 µl, 0.8 mmol). The mixture was heated at 80ºC for 17 h and the volatiles were evaporated. The residue was re-dissolved in acetonitrile (15 ml) and heating was continued for 24 h. The mixture was filtered and concentrated. Purification was achieved using HPLC system 3.

Yield: 23%

LC-MS (Method 1): Rt 12.09 min, m/z 1124.30 [MH^+]

Example 21

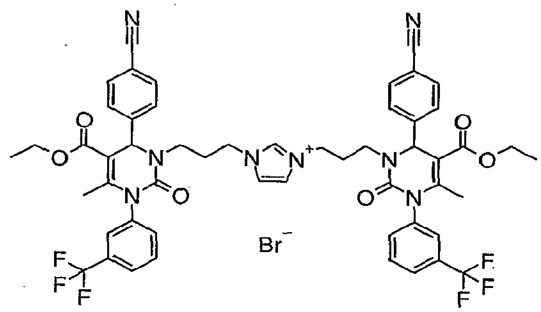


Intermediate 10 (70 mg, 0.130 mmol) and Intermediate 12 (72 mg, 0.130 mmol) were dissolved in acetonitrile (5 ml) and the solution was heated at 80°C for 40 h. Evaporation of the solvent gave a residue that was purified by HPLC system 1.

Yield: 49 mg (34%)

LC-MS (Method 1): Rt 11.03 min, 1007.06 m/z [M^+]

Example 22

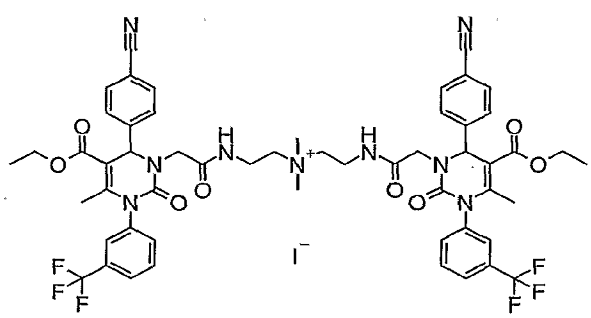


Example 22 was prepared in a similar manner from Intermediate 13 and Intermediate 11 and purified using HPLC system 3.

Yield: 8%

LC-MS (Method 1): Rt 10.85 min, m/z 1007.22 [MH^+]

Example 23



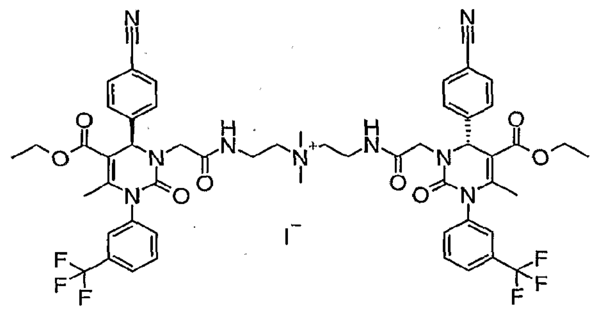
Example 10 (38 mg, 0.035 mmol) and iodomethane (1 ml) were dissolved in acetonitrile (5 ml). $NaHCO_3$ (18 mg, 0.214 mmol) was added and the mixture was heated at 80°C for 25 h. A further portion of iodomethane (1 ml) was added and heating was continued for a

further 17 h, after which time the reaction mixture was allowed to cool and the solvent and excess iodomethane were evaporated. The residue was purified using HPLC system 1.

Yield: 11 mg (26%)

LC-MS (Method 1): Rt 10.40 min, 1070.07 m/z [M$^+$]

Example 24

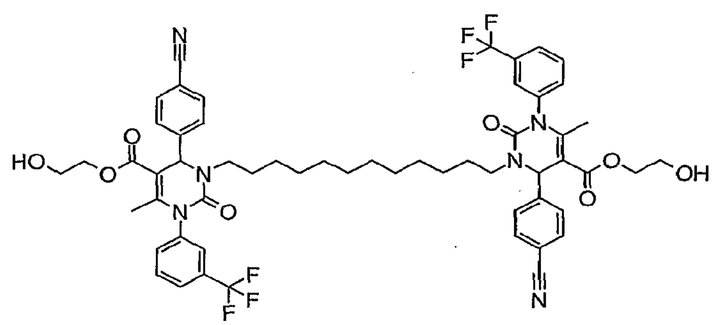


Example 24 was prepared in a similar manner from Example 11 and purified using HPLC system 3.

Yield: 67%

LC-MS (Method 1): Rt 10.15 min, m/z 1070.26 [M$^+$]

Example 25

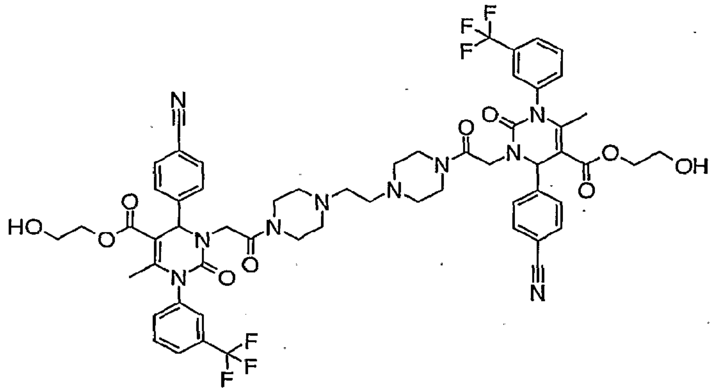


Intermediate 18 (100 mg, 0.095 mmol) was dissolved in acetonitrile (3 ml) and 1M aqueous HCl was added until the mixture became opaque. Acetonitrile was added dropwise until the solution became clear. After 1 h the product was extracted into EtOAc and the solution was washed with brine and dried (Na_2SO_4). Evaporation gave a residue which was purified using HPLC system 4.

Yield: 6.5 mg (6%)

LC-MS (Method 1): Rt 14.97 min, m/z 1057.30 [MH$^+$]

Example 26

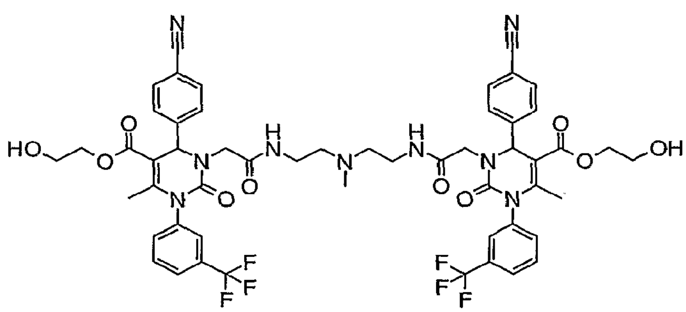


Example 26 was prepared from Intermediate 8 and Intermediate 17 by a method similar to that used for the synthesis of Example 13 The product was purified using HPLC system 4.

Yield: 17%

LC-MS (Method 1): Rt 9.34 min, m/z 1169.24 [MH^+]

Example 27

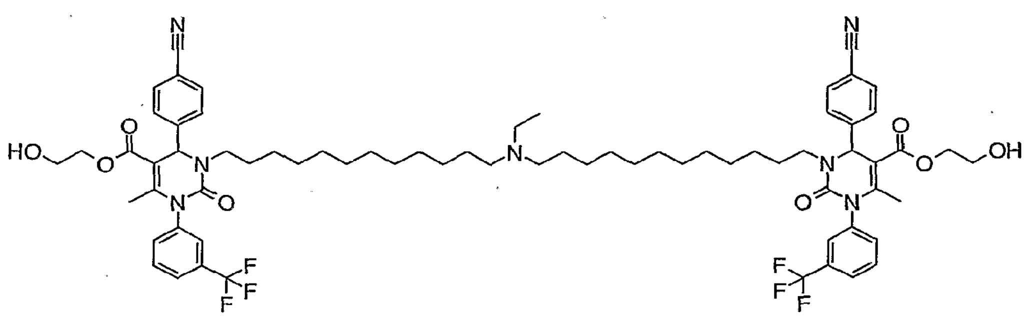


Example 27 was prepared from Intermediate 17 and *N*-methyl-2,2′-diaminoethylamine by a method similar to that used for the synthesis of Intermediate 38. The product was purified using HPLC system 4.

Yield: 14%

LC-MS (Method 1): Rt 9.42 min, m/z 1088.18 [MH^+]

Example 28

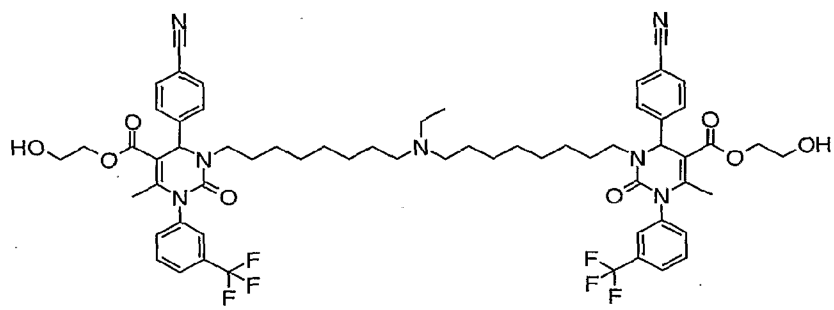


Intermediate 19 (40 mg, 0.058 mmol) was dissolved in acetonitrile (4 ml) and 1M aqueous HCl (~2 ml) was added until the mixture went opaque. Acetonitrile was added drop wise until the solution was clear. After 1 h diethyl ether was added. The organic layer was separated, washed with water and brine, and dried (Na_2SO_4). The solvent was evaporated and the residue was re-dissolved in acetonitrile (4 ml). $NaHCO_3$ (15 mg, 0.17 mmol) and 2M ethylamine in THF (58 ml, 0.12 mmol) were added and the mixture was heated at 80ºC for 24 h. After filtering, the solvent was removed and the product was purified using HPLC system 3.

Yield: 10 mg (27%)

LC-MS (Method 1): Rt 12.20 min, m/z 1268.41 [MH^+]

Example 29

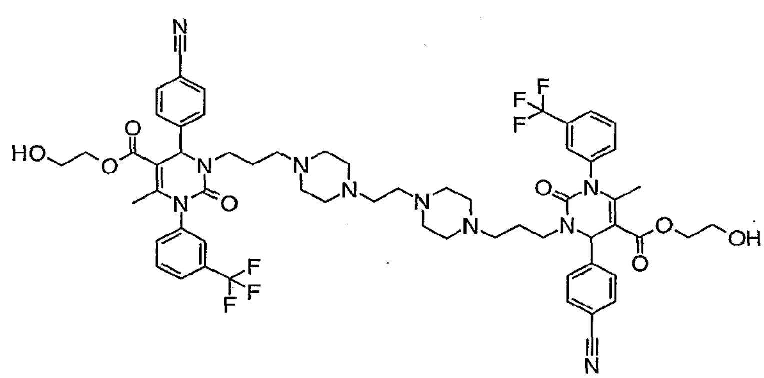


Example 29 was prepared from Intermediate 23 by a method similar to that used for the synthesis of Example 28. The product was purified using HPLC system 3.

Yield: 69%

LC-MS (Method 1): Rt 10.70 min, m/z 1156.38 [MH^+]

Example 30

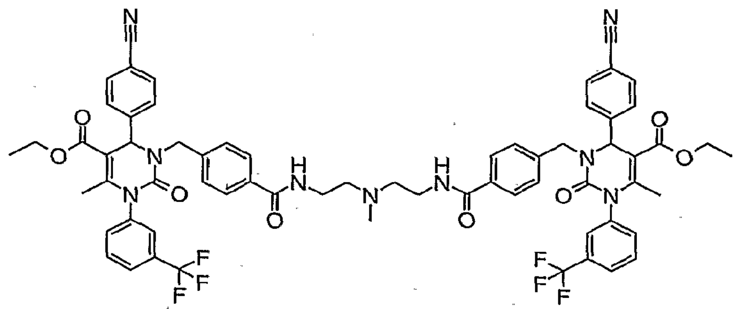


Example 30 was prepared from Intermediate 22 by a similar method to that used in the synthesis of Example 25. The product was purified by HPLC system 3.

Yield: 36%

LC-MS (Method 1): Rt 8.10 min, m/z 1169.38 [MH^+]

Example 31

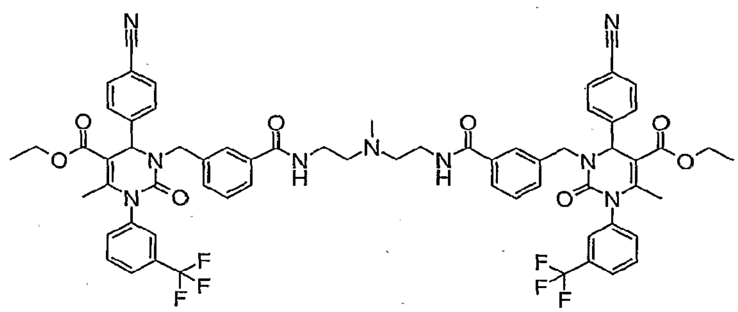


Example 31 was prepared from Intermediate 25 and *N*-methyl-2,2′-diaminoethylamine by a method similar to that used for the synthesis of Intermediate 38. The product was purified using HPLC system 1.

Yield: 61%

LC-MS (Method 1): Rt 10.76 min, m/z 1208.18 [MH^+]

Example 32

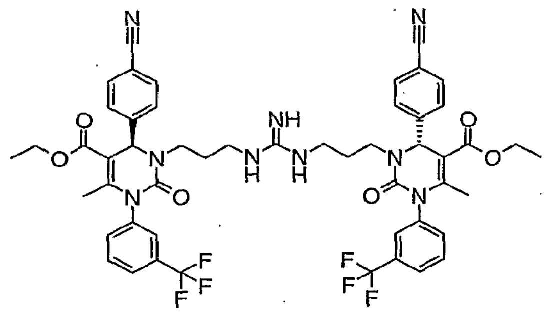


Example 32 was prepared from Intermediate 27 and *N*-methyl-2,2′-diaminoethylamine by a method similar to that used for the synthesis of Intermediate 38. The product was purified using HPLC system 1.

Yield: 53%

LC-MS (Method 1): Rt 10.93 min, m/z 1208.20 [MH^+]

Example 33

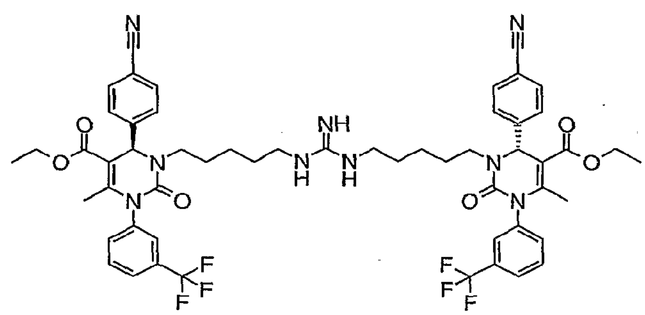


Intermediate 32 (263 mg, 0.27 mmol) was dissolved in IMS (10 ml) and iodomethane (379 ml, 2.67 mmol) was added. The solution was allowed to stand for 24 h and the volatiles were evaporated. The residue was dissolved in 2M NH_3 in EtOH (10 ml) and heated at 50ºC in a sealed tube for 50 h. The solvent was removed and the product was purified by HPLC system 3.

Yield: 24 mg (9%)

LC-MS (Method 1): Rt 10.84 min, m/z 998.19 [MH^+]

Example 34

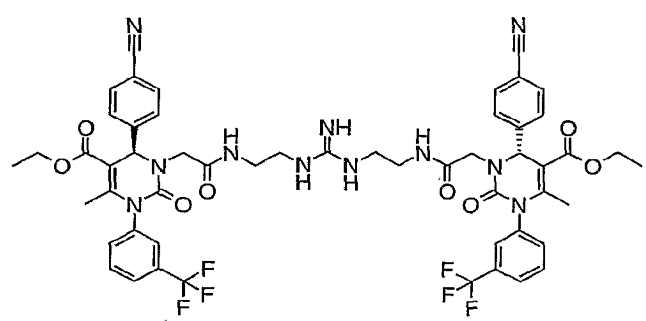


Example 34 was prepared from Intermediate 33 by a method similar to that used for the synthesis of Example 33. The product was purified using HPLC system 1.

Yield: 44%

LC-MS (Method 1): Rt 11.28 min, m/z 1054.26 [MH^+]

Example 35

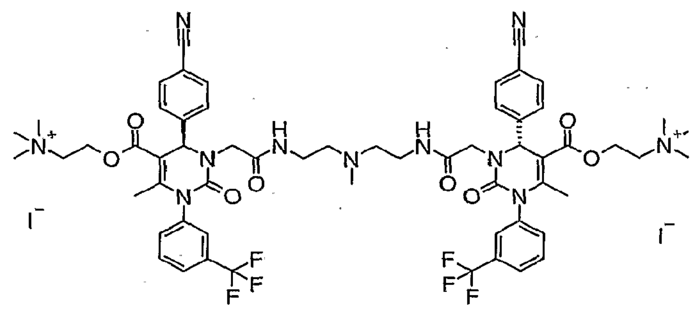


Intermediate 35 (150 mg, 0.127 mmol) was dissolved in MeOH (10 ml) and a solution of K_2CO_3 (235 mg, 1.71 mmol) in water (4 ml) was added. The mixture was stirred at RT for 30 min and then diluted with EtOAc (60 ml) and water (50 ml). The organic layer was separate and washed with brine. After drying (Na_2SO_4) the solvent was evaporated and the residue was purified using HPLC system 3.

Yield: 50 mg (36%)

LC-MS (Method 1): Rt 10.20 min, m/z 1084.23 [MH^+]

Example 36

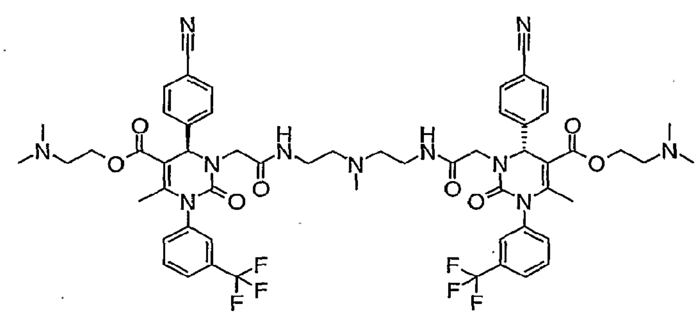


Intermediate 39 (150 mg, 0.15 mmol) was dissolved in DCM (4 ml) and hydroxyethyltrimethyl-ammonium iodide (173 mg, 0.75 mmol), EDC (86 mg, 0.45 mmol) and DMAP (55 mg, 0.45 mmol) were added. The mixture was stirred at RT for 7 h and water (4 ml) was added. The aqueous layer was separated and evaporated. Purification was achieved using HPLC system 3.

Yield: 35 mg (16%)

LC-MS (Method 1): Rt 6.78 min, m/z 585.80 [M^{2+}]/2

Example 37

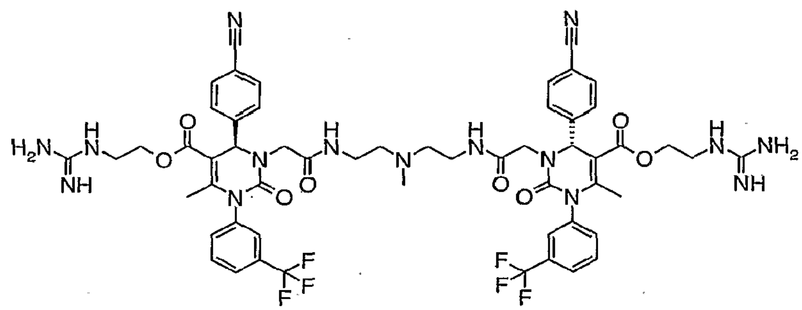


Intermediate 39 (150 mg, 0.15 mmol) was dissolved in DCM (4 ml) and *N,N*-dimethylethanolamine (67 mg, 0.75 mmol), EDC (86 mg, 0.45 mmol) and DMAP (55 mg, 0.45 mmol) were added. The mixture was stirred at RT for 50 h and water (4 ml) was added. After shaking, the organic layer was separated and evaporated. Purification was achieved using HPLC system 3.

Yield: 36 mg (21%)

LC-MS (Method 1): Rt 6.84 min, m/z 1142.37 [MH^+]

Example 38

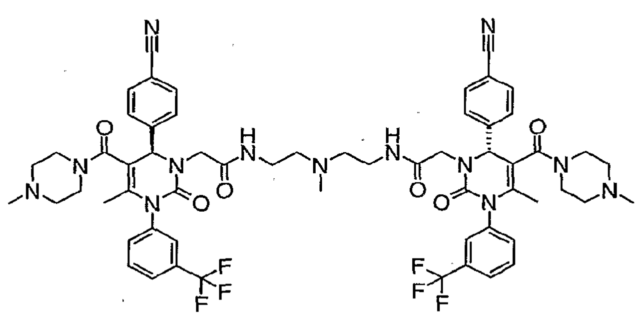


Intermediate 39 (150 mg, 0.15 mmol) was dissolved in DCM (4 ml) and 1,3-di-^{t}Boc-2-(2-hydroxyethyl)-guanidine (227 mg, 0.75 mmol), EDC (86 mg, 0.45 mmol) and DMAP (55 mg, 0.45 mmol) were added. The mixture was stirred at RT for 18 h the solution was diluted with DCM (8 ml) and washed with water (4 ml). TFA (2 ml) was added and the solution was allowed to stand at RT for 5 h after which time it was washed with sat. aqueous $NaHCO_3$ (5 ml) and evaporated. The residue, and any residue which did not dissolve in either DCM or water, was dissolved in 20% acetonitrile in water and purified by HPLC system 3.

Yield: 45 mg (26%)

LC-MS (Method 1): Rt 6.74 min, m/z 585.87 [MH_2^{2+}]/2

Example 39

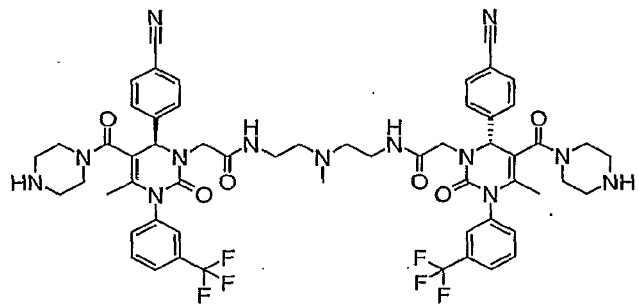


Example 39 was prepared from Intermediate 43 and *N*-methyl-2,2′-diaminoethylamine by a method similar to that used for the synthesis of Intermediate 38. The product was purified using HPLC system 3.

Yield: 37%

LC-MS (Method 1): Rt 5.72 min, m/z 1164.17 [MH^+]

Example 40

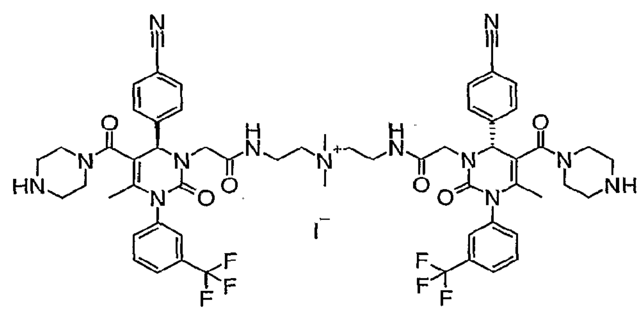


Intermediate 45 (191 mg, 0.143 mmol) was dissolved in DCM (15 ml) and TFA (5 ml) was added. The reaction was allowed to stand at RT for 1.5 h before evaporation of the volatiles. The product was purified using HPLC system 3.

Yield: 67 mg (41%)

LC-MS (Method 1): Rt 5.64 min, m/z 1136.14 [MH^+]

Example 41

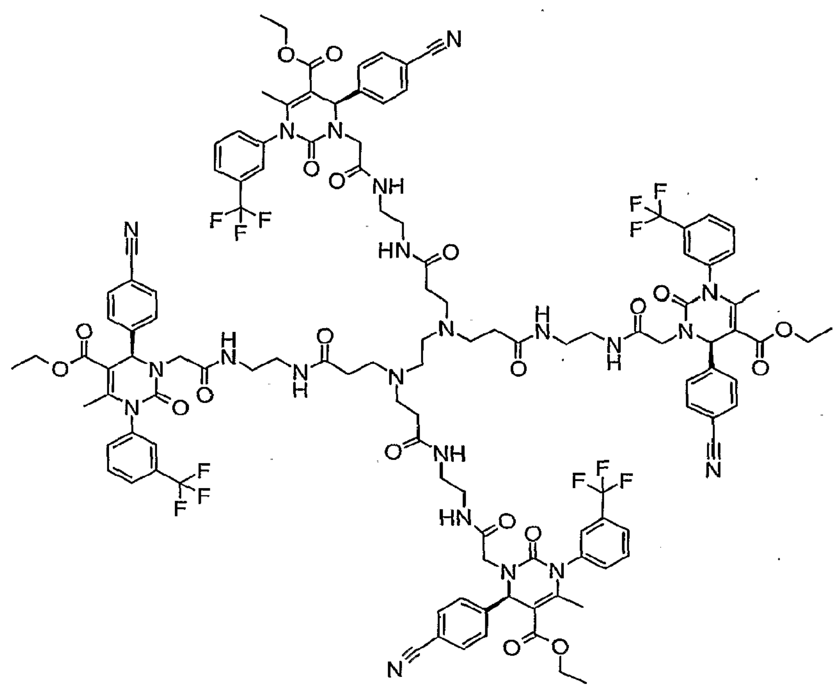


Intermediate 45 (191 mg, 0.143 mmol) was dissolved in DCM (20 ml) and iodomethane (5 ml) was added. The solution was allowed to stand at RT for 48 h. The volatiles were evaporated and the residue dissolved in 25% TFA in DCM (20 ml). After 1 h, evaporation gave a residue which was purified by HPLC system 3.

Yield: 35 mg (19%)

LC-MS (Method 1): Rt 5.65 min, m/z 1150.15 [M^+]

Example 42

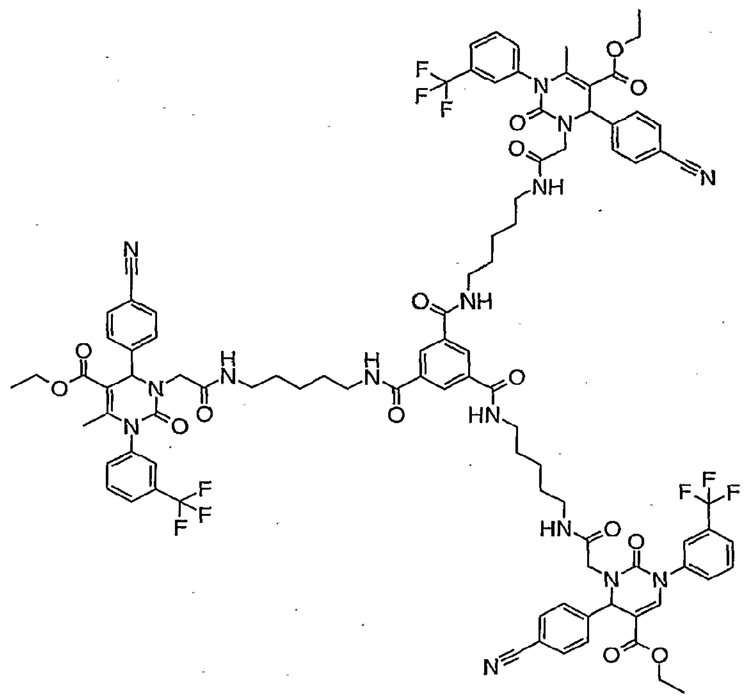


Example 42 was prepared from Intermediate 7 and 0.25 equivalents of G_0 PAMAM dendrimer using a similar procedure to that used in the synthesis of Intermediate 38. The product was purified using HPLC system 3.

Yield: 19%

LC-MS (Method 1): Rt 12.32 min, m/z 2394.49 [MH^+]

Example 43

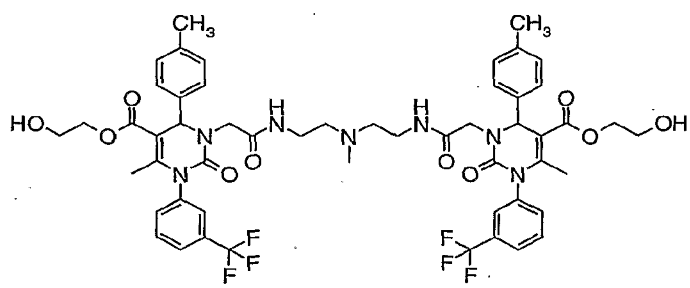


Intermediate 50 (168 mg, 0.295 mmol), benzenetricarbonyl trichloride (13 mg, 0.05 mmol) and DIPEA (0.2 ml) were dissolved in DCM (10 ml) and the mixture was stirred at RT for 40 min. A further quantity of benzenetricarbonyl trichloride (5 mg, 0.019 mmol) was added and the reaction was stirred for a further 48 h. The solvent was evaporated and the residue partially solidified. The product was isolated using HPLC system 2.

Yield: 90 mg (49%)

LC-MS (Method 1): Rt 14.45 min, m/z 1871.31 [MH^+]

Example 44

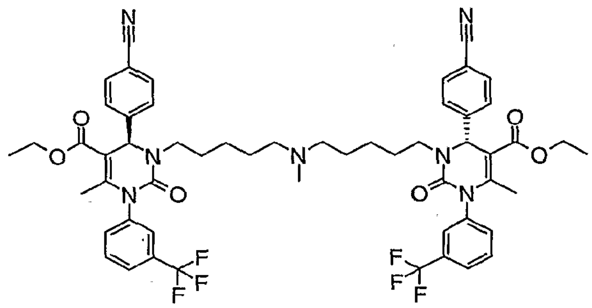


Intermediate 48 (150 mg, 0.118 mmol) was dissolved in EtOAc (15 ml) and acetic acid (2 drops) was added, followed by a spatula of $Pd(OH)_2$ on carbon. The reaction mixture was stirred under an atmosphere of hydrogen for 17 h. A further spatula of catalyst was added and stirring was continued for a further 5 h. The mixture was filtered and the solvent was evaporated. The product was purified by HPLC system 2.

Yield: 32 mg (25%)

LC-MS (Method 1): Rt 9.77 min, m/z 1066.22 [MH$^+$]

Example 45

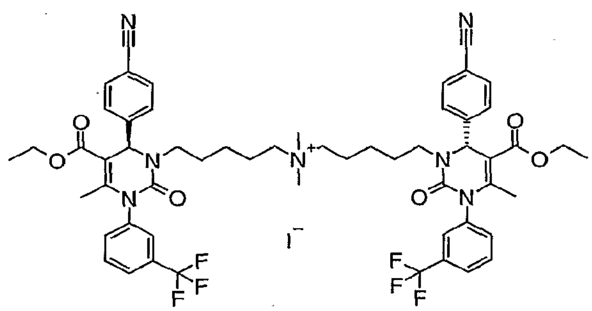


Example 45 was prepared from Intermediate 40 and 2M methylamine in THF by a method similar to that used for the synthesis of Example 19. The product was purified using HPLC system 3.

Yield: 22%

LC-MS (Method 1): Rt 11.08 min, m/z 1026.16 [MH$^+$]

Example 46

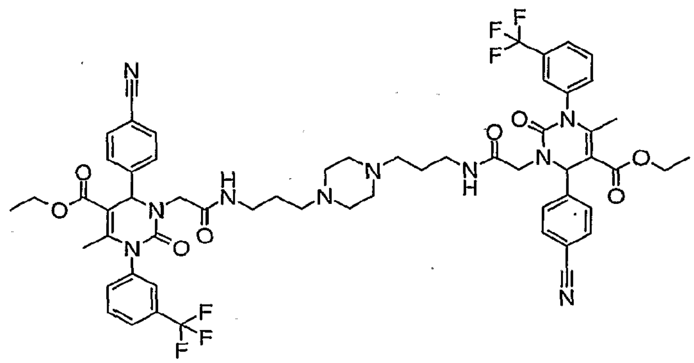


Example 45 (50 mg, 0.049 mmol) was dissolved in DCM (5 ml) and treated with iodomethane (1.5 ml). The solution was allowed to stand for 24 h. The volatiles were evaporated and the product was purified using HPLC system 3.

Yield: 24 mg (42%)

LC-MS (Method 1): Rt 11.27 min, m/z 1040.27 [M$^+$]

Example 47

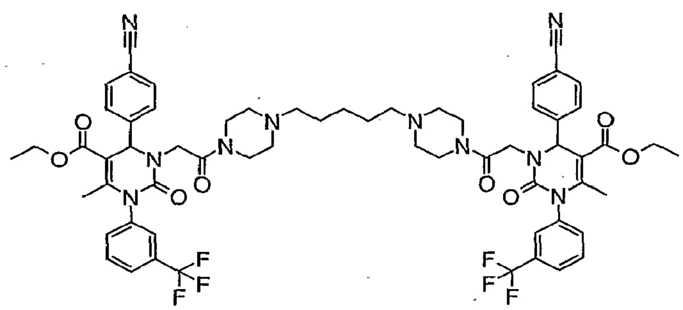


Example 47 was prepared from Intermediate 6 and Intermediate 59 by a procedure similar to that used for the synthesis of Intermediate 38. The product was purified by column chromatography using 0-5% EtOH in DCM. The material obtained was evaporated and purified further by preparative HPLC system 3.

Yield: 23%

LC-MS (Method 1): Rt 9.64 min, 1139.22 m/z [MH^+]

Example 48

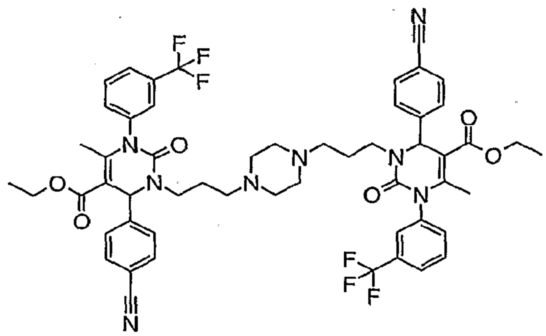


Intermediate 48 was prepared from Intermediate 6 and Intermediate 60 by a procedure similar to that used for the synthesis of Intermediate 38.

Yield: 51%

LC-MS (Method 1): Rt 8.61 min, 1179.37 m/z [MH^+]

Example 49

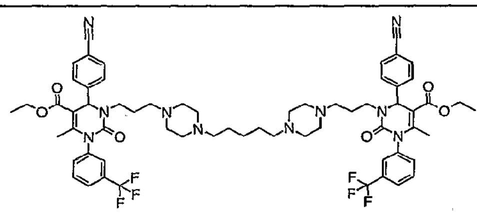
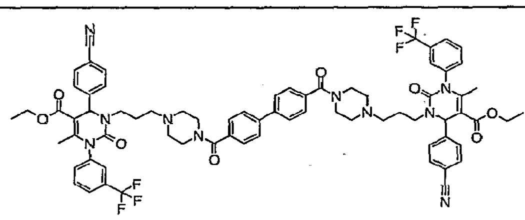


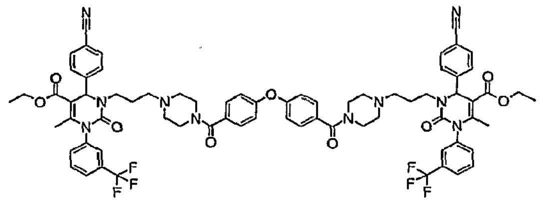
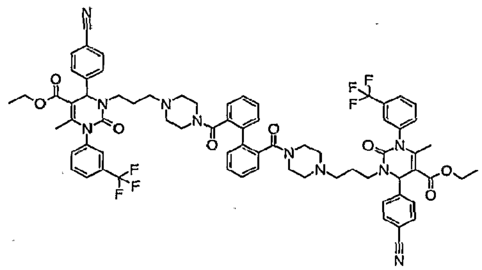
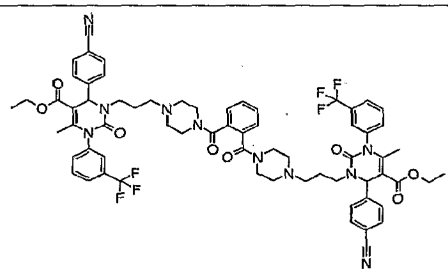
To a solution of Intermediate 68 (200 mg, 0.412 mmol) in DCE (8 ml) was added piperazine (17 mg, 0.206 mmol), powdered molecular sieves and sodium triacetoxyborohydride (98 mg, 0.464 mmol). The reaction mixture was stirred overnight then diluted with DCM and filtered before the solvent was removed. The crude material was purified by preparative HPLC system 3.

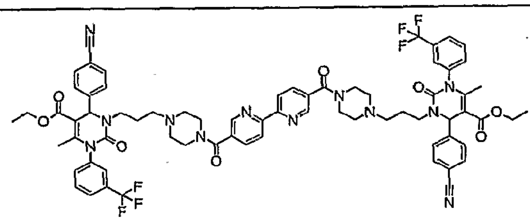
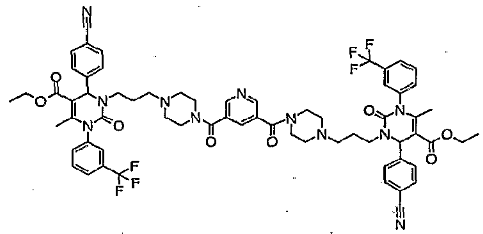
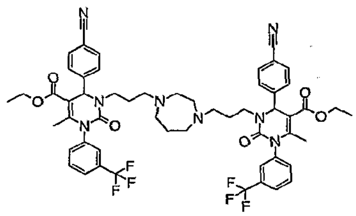
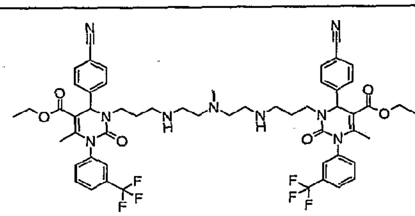
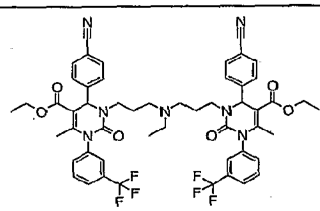
Yield: 35 mg (17%)

LC-MS (Method 1): Rt 10.69 min, 1025.30 m/z [MH^+]

In analogy to the procedure for Example 49, the following compounds were prepared from Intermediate 67 and 0.5 equivalents of an amine or diamine (respectively Intermediates 60 to 66, homopiperazine, *N*-(2-aminoethyl)-*N*-methylethane diamine and ethylamine).

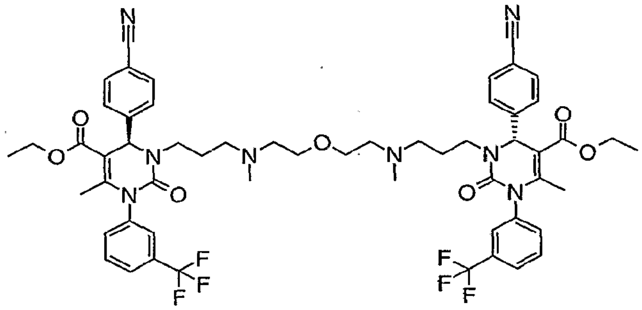
Example	Structure	Reaction time (h)	Yield (%)	Rt (min) (Method 1)	Mass $[MH]^+$
Example 50		18	20	8.57	1179.42
Example 51		18	11*	9.02	1317.19

Example 52		18	16*	9.01	1333.28
Example 53		18	10*	9.12	1317.22
Example 54		18	28*	8.80	1241.33

Example 55		18	25*	8.77	1319.32
Example 56		18	18*	8.65	1242.15
Example 57		18	25*	9.38	1039.27
Example 58		18	ND	8.84	1056.23
Example 59		18	35*	10.75	984.21

*yield based on purification of half of the crude mixture

Example 60



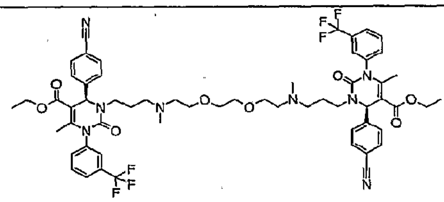
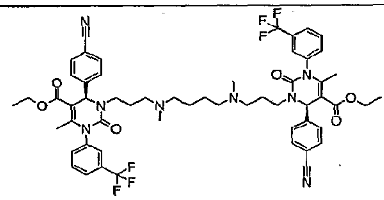
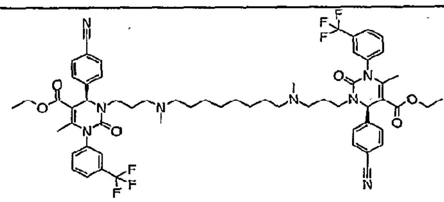
To a solution of Intermediate 70 (200 mg, 0.412 mmol) in THF (4 ml) under nitrogen was

added 1,5-bis(methylamino)-3-oxapentane (22 mg, 0.166 mmol) and PS-Triacetoxyborohydride (358 mg, 0.8 mmol). The reaction mixture was shaken overnight then filtered and the solvent removed. The crude material was purified by HPLC system 3.

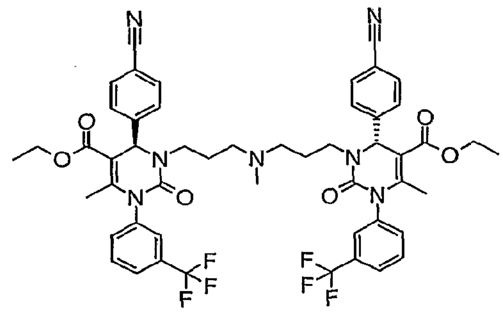
Yield: 45 mg (25%)

LC-MS (Method 1): Rt 8.94 min, 1071.28 m/z [MH^+]

In analogy to the procedure for Example 60 the following compounds were prepared from Intermediate 70 and 0.5 equivalents of a diamine:

Example	Structure	Diamine	Reaction time (h)	Yield (%)	Rt (min) (Method 1)	Mass $[MH]^+$
Example 61		1,8-bis(methylamino)-3,6-dioxaoctane	18	24	8.92	1115.29
Example 62		*N,N'*-dimethylbutane-1,4-diamine	18	29	8.85	1055.28
Example 63		*N,N'*-dimethyloctane-1,8-diamine	18	33	9.07	1111.33

Example 64

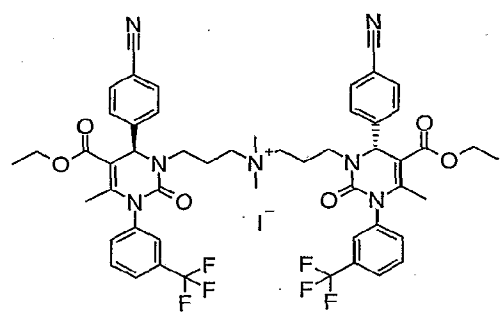


To a solution of Intermediate 11 (250 mg, 0.455 mmol) in THF (3 ml) was added $NaHCO_3$ (115 mg, 1.37 mmol) and 2M methylamine in THF (250 µl, 0.455 mmol). The reaction mixture was heated at 80°C for 5 h. A further amount of methylamine (500 µl) was added and heating continued overnight. The volatiles were evaporated and THF (3 ml) and $NaHCO_3$ (115 mg) were added. Heating at 80°C was resumed for 18 h. Evaporation gave crude material which was purified by HPLC system 3.

Yield: 55 mg (35%)

LC-MS (Method 1): Rt 10.73 min, 970.18 m/z [MH^+]

Example 65

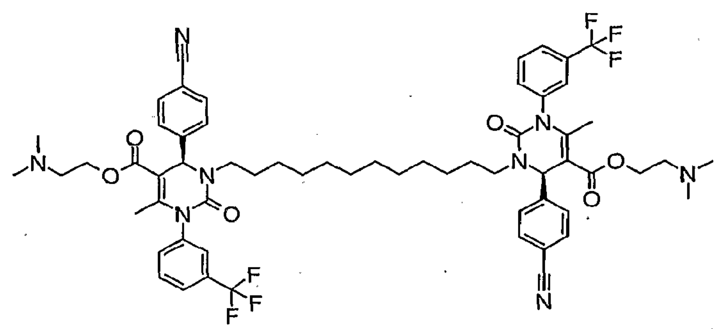


Example 65 was prepared from Example 64 using a similar procedure to that used for the synthesis of Example 46. The crude material was purified by HPLC system 3.

Yield: 69%

LC-MS (Method 1): Rt 10.73 min, 985.08 m/z [M^+]

Example 66

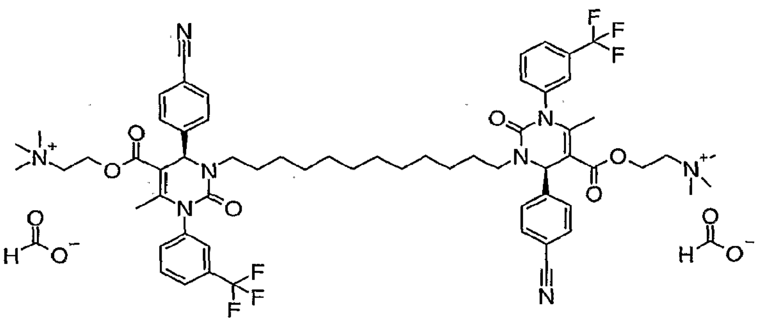


Example 66 was prepared from Intermediate 71 and 0.5 equivalents of 1,9-dibromododecane using a method similar to that used for the preparation of Intermediate 10. The product was purified by HPLC system 3.

Yield: 25%

LC-MS (Method 1): Rt 9.04 min, 1111.39 m/z [MH^+]

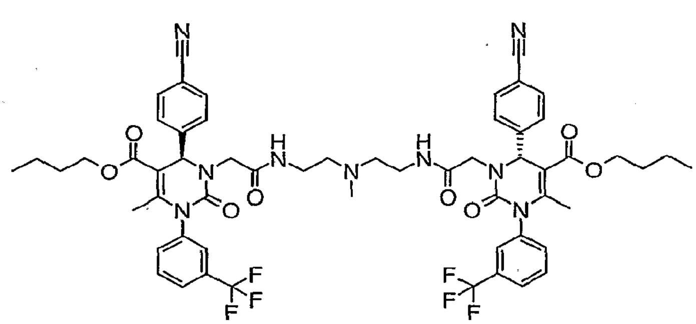
Example 67



Example 66 (43 mg, 0.039 mmol) was dissolved in DCM (5 ml) and iodomethane (1ml) was added. The solution was stirred for 17 h then the solvent was removed and the crude material was purified by HPLC system 3.

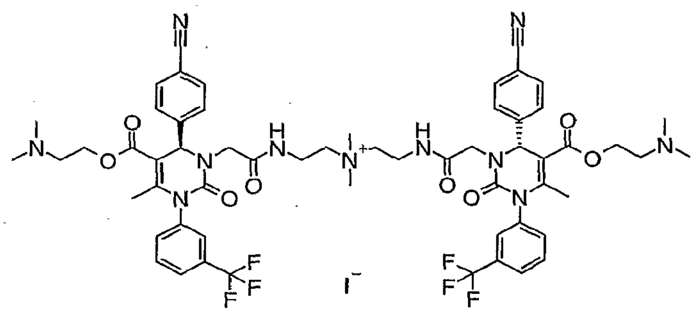
LC-MS (Method 1): Rt 9.22 min, 570.80 m/z [M^{2+}]/2

Example 68



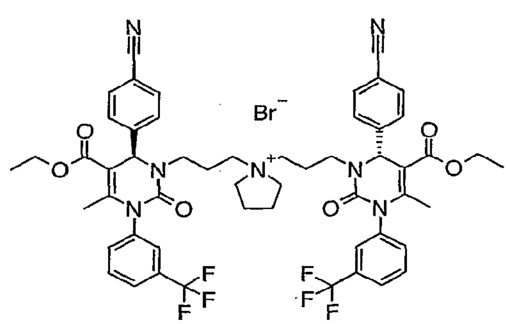
Example 68 was prepared from Intermediate 72 and 0.5 equivalents of *N*-methyl-2,2′-diaminoethylamine by a method similar to that used for the synthesis of Intermediate 38.
Yield: 42%
LC-MS (Method 1): Rt 11.36 min, 1112.28 m/z [MH^+]

Example 69



Example 69 was prepared from Intermediate 75 and dimethylaminoethanol by a similar procedure to that used in the synthesis of Example 36. The product was purified by chromatography on an Isolute Si II cartridge (10 g) using EtOAc; 5% of 2M NH_3 in MeOH in EtOAc; 100% of 2M NH_3 in MeOH then 5% conc. HCl (37% aqueous solution) in MeOH. The material isolated was taken up in acetonitrile and water then filtered and the filtrate evaporated then purified using HPLC system 3.
Yield: 52 mg (9%)
LC-MS (Method 1): Rt 6.57min, 1156.26 m/z [M^+]

Example 70



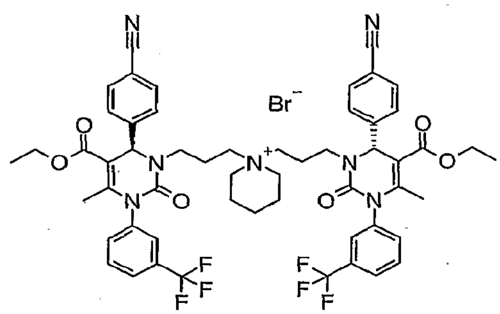
Intermediate 11 (100 mg, 0.182 mmol) was dissolved in acetonitrile (3 ml) and $NaHCO_3$ (76 mg, 0.91 mmol) and pyrrolidine (45 μl, 0.545 mmol) were added. The reaction was stirred for 18 h then filtered and evaporated. The residue was re-dissolved in DCM and

washed with water and the solvent removed to afford monosubstituted pyrrolidine. This material was dissolved in acetonitrile (3 ml) then Intermediate 11 (100 mg, 0.182 mmol) was added, followed by $NaHCO_3$ (31 mg, 0.364 mmol) and the mixture stirred at RT for 30 h then at 40°C for 6 h. A further amount of Intermediate 11 (145 mg, 0.264 mmol) was added and the reaction heated at 80°C for 30 h. The crude material was filtered then evaporated and purified using HPLC system 3.

Yield: 103 mg (52%)

LC-MS (Method 1): Rt 10.83 min, 1010.09 m/z [M^+]

Example 71

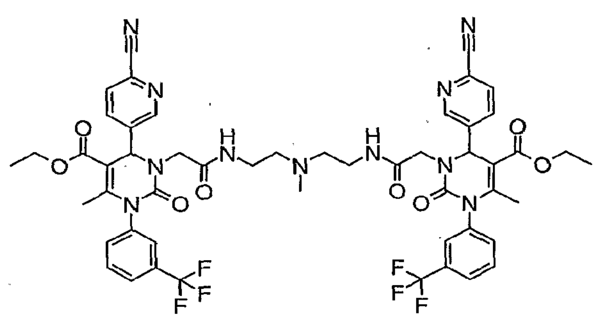


Example 71 was prepared from Intermediate 11 and piperidine using a similar method to that used in the synthesis of Example 70, except that the reaction was heated at 80°C for 48 h in second step of procedure.

Yield: 34%

LC-MS (Method 1): Rt 10.91 min, 1024.22 m/z [M^+]

Example 72

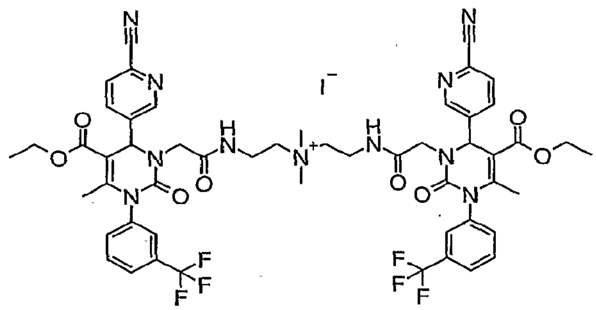


Example 72 was prepared from Intermediate 77 and 0.5 equivalents of *N*-methyl-2,2′-diaminoethylamine according to a method similar to that described for described for Intermediate 38.

Yield: 39%

LC-MS (Method 1): Rt 9.82 min, 1058.19 m/z [MH^+]

Example 73

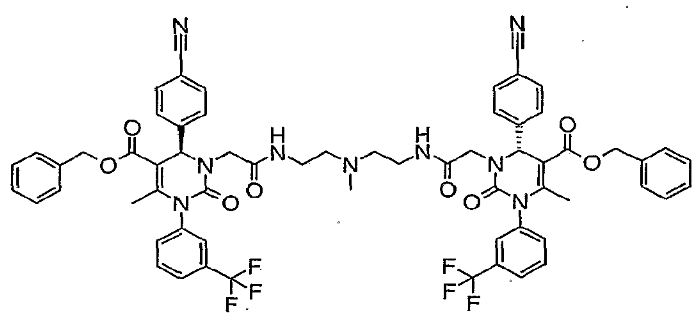


Example 72 (25 mg, 0.024 mmol) was dissolved in acetonitrile (3 ml), iodomethane (1 ml) was added and the reaction stirred at 80°C overnight. The solvent was removed and the crude material purified using HPLC system 3.

Yield: 5 mg (20%)

LC-MS (Method 1): Rt 9.77 min, 1072 [M^+]

Example 74

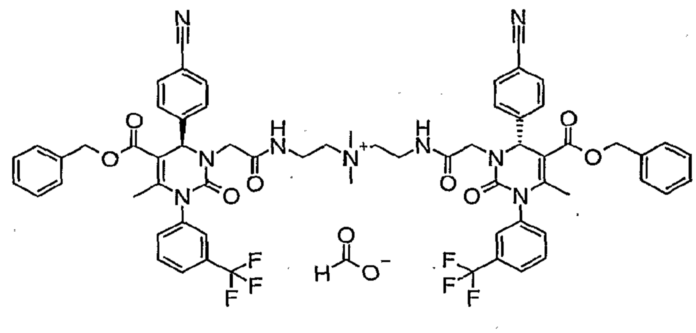


Example 74 was prepared from Intermediate 79 and 0.5 equivalents of *N*-methyl-2,2′-diaminoethylamine according to a method similar to that described for described for Intermediate 38. Purification was achieved using HPLC system 3.

Yield: 29%

LC-MS (Method 1): Rt 11.25 min, 1180.10 [MH^+]

Example 75

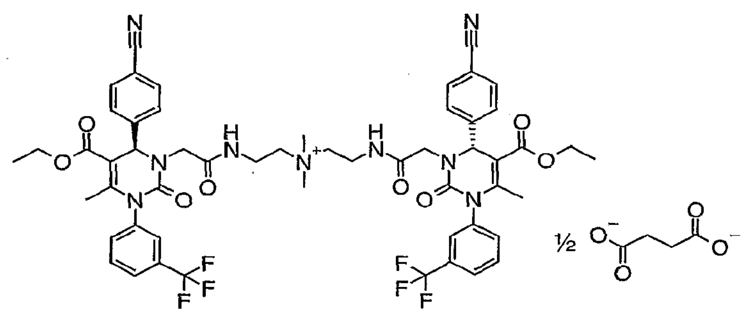


Example 74 (49 mg, 0.0415 mmol) was dissolved in acetonitrile (5 ml) and $NaHCO_3$ (7 mg, 0.083 mmol) and iodomethane (3 drops) added. The mixture was stirred at RT for 4 h then a further amount of iodomethane (3 drops) was added and the mixture stirred for 17 h. The crude material was filtered then evaporated then purified by using HPLC system 3.

Yield: 27 mg (54%)

LC-MS (Method 1): Rt 11.20min, 1194.23 m/z [M^+]

Example 76



Succinic acid (4.9 mg, 0.042 mmol) was added to a stirred suspension of silver (I) oxide (9.67 mg, 0.042 mmol) in de-ionised water (2 ml). The mixture was stored at RT for 3 h with occasional warming. The cloudy solution was treated with Example 24 (100 mg, 0.083 mmol) and the resulting mixture was stirred at RT for 16 h. After this time the mixture was centrifuged (15ºC, 13000 r.p.m.) for 1 h and the supernatant was removed and freeze-dried to give the hemi-succinate salt as a beige solid.

Yield: 64 mg (64%)

LC-MS (Method 1): Rt 10.25 min, 1070.11 m/z [M+]

Melting point: 138-143ºC

Elastase inhibition assays

Various compounds of the invention were tested for their inhibitory activity

towards HNE.

Fluorescent peptide substrate

Assays were performed in 96-well plates at a total assay volume of 100µl. The final concentration of the enzyme (human leukocyte elastase, Sigma E8140) was 0.00036 units/well. A peptide substrate (MeO-Suc-Ala-Ala-Pro-ValAMC, Calbiochem #324745) was used, at the final concentration of 100µM. The final concentration of DMSO was 1% in the assay buffer (0.05M Tris.HCl, pH 7.5, 0.1M NaCl; 0.1M CaCl2; 0.0005% brij-35).

The enzymatic reaction was started by adding the enzyme. The enzymatic reaction was performed at RT and after 30mins stopped by adding 50µl soybean trypsin inhibitor (Sigma T-9003) at a final concentration of 50µg/well. Fluorescence was read on the FLEXstation (Molecular Devices) using 380 nm excitation and 460 nm emission filters. The potency of the compounds was determined from a concentration series of 10 concentrations in range from 1000 nM to 0.051nM. The results are means of two independent experiments, each performed in duplicate.

The compounds tested were shown to have IC_{50} values for HNE in the range 1-1000nM.

Using Fluorescently labelled elastin

Assays were performed in 96-well plate at a total assay volume of 100µl. The final concentration of the enzyme (human leukocyte elastase, Sigma E8140) was 0.002 units/well. Fluorescently labelled, solubilised elastin from bovine neck ligament (Molecular Probes, E-12056) was used at the final concentration of 15µg/ml. The final concentration of DMSO was 2.5% in the assay buffer (0.1M Tris-HCL,pH8.0, containing 0.2mM sodium azide).

The enzymatic reaction was started by adding the enzyme. The enzymatic reaction was performed at RT and read after 120 minutes. Fluorescence was read on the FLEXstation (Molecular Devices) using 485 nm excitation and 530 nm emission filters. The potency of the compounds was determined from a concentration series of 10 concentrations in range from 25000nM to 1nM. The results are means of two independent experiments, each performed in duplicate.

The compounds tested were shown to have IC_{50} values for HNE in the range 1-1000nM.

Elastase Selectivity Assays

Selectivity for elastase inhibition was determined by testing the compounds against a panel of 6 proteases: plasmin, thrombin, cathepsin G, proteinase 3, trypsin,

chymotrypsin (all sourced from Sigma, cat. No. P1867, T1063, C4428, P0615, T6424, C8949 respectively). Assays were performed in 96-well plate at a total assay volume of 100μl. A common, generic substrate was used for all proteases: fluorescently labelled casein (Molecular Probes, E-6639), at the final concentration of 20μg/ml (Cathepsin G and Chymotrypsin), 10μg/ml (Plasmin and Thrombin) or 5μg/ml (Proteinase 3 and Trypsin). The final concentration of the substrate was close to the respective K_m values as determined for this substrate. The final concentration of DMSO was 5% in the assay buffer (0.05M Tris.HCl, pH 7.5, 0.1M NaCl; 0.1M CaCl2; 0.0005% brij-35). The enzymatic reaction was started by adding the enzyme. The enzymatic reaction was performed at RT for 60min. Fluorescence was read on the FLEXstation (Molecular Devices) using 589 nm excitation and 617 nm emission filters. The potency of the compounds was determined from a concentration series of 8 concentrations in range from 500μM to 0.2μM. The results are means of two independent experiments, each performed in duplicate.

The compounds tested showed selectivities for a range of proteases from1 to >300 fold.

Membrane Bound Elastase

Blood was collected from healthy human volunteers. PMNs were isolated by density centrifugation on ficol and red blood cells lysed hypotonically Cells were fixed with paraformaldehyde / gluteraldehyde and washed by centrifugation.

Compounds were made up in HBSS containing and incubated for 5 minutes at 37°C with cells. Fluorogenic AAPV substrate (Calbiochem #324745) was added to each well to make 100μl final volume and the plate read using a Spectramax Gemini Ex 380nm Em 460 for 30minutes at 37°C.

Compounds of the invention examined in this assay were found to exhibit IC_{50} values of less than 100nM, preferably less than 20nM.

Intracellular Elastase (controlled with lysed cell elastase)

PMNs were isolated as described previously. PMNs were added to 96-well polypropylene plates and DMSO or compound added to each well to give 150μl volume. The plate was incubated at 37°C for 30minutes. Cells were washed by centrifugation and lysed with HBSS containing 0.04% triton. Cell debris was pelleted and the supernatant transferred to a fresh pate, with compounds or DMSO. Fluorogenic AAPV substrate was added to all wells and the plate was read using a Spectramax Gemini Ex 380nm Em 460 for 30minutes at 37°C.

Neutrophil Released Elastase Activity Assay (Human, Mouse, Guinea Pig)

Generation of Released Neutrophil Elastase, from Guinea Pigs

Guinea pigs were treated with an LPS aerosol. Animals were left for 4 hours, euthanized and the lungs lavaged to recover PMN. Bronchoalveolar lavage fluid (BAL) was spun at 400g for 10minutes and the cells resuspended in HBSS. 10μM cytocholasin B was added to the cell suspension and incubated at 370C for 5 minutes after which 1μM fMLP was added for a further 5 minutes. Cells were centrifuged at 400g for 10 minutes. 'Elastase rich supernatant' was transferred to a fresh tube.

Generation of Released Neutrophil Elastase, from Mice

Mice were anaesthetised and treated with LPS i.n. Animals were left for 4 hours, euthanized and the lungs lavaged to recover PMN. Bronchoalveolar lavage fluid (BAL) was centrifuged at 400g for 10minutes and the cells resuspended in 1ml of HBSS. 10μM cytocholasin B was added to the cell suspension and incubated at 370C for 5 minutes after which 1μM fMLP was added for a further 5 minutes. Cells were centrifuged at 400g for 10 minutes. 'Elastase rich supernatant' was transferred to a fresh tube.

Generation of Human Released Neutrophil Elastase, from Humans

Human PMN were isolated as described previously. 10μM cytocholasin B was added to the cell suspension and incubated at 370C for 5 minutes after which 1μM fMLP was added for a further 5 minutes. Cells were centrifuged at 400g for 10 minutes. 'Elastase rich supernatant' was transferred to a fresh tube.

To a clear bottomed 96-well plate compounds were added and incubated for 5 minutes at 37)C with 'elastase rich' supernatant. Fluorogenic AAPV substrate was added to all wells and the plate read using a Spectramax Gemini Ex 380nm Em 460 for 30 minutes at 37°C. For comparison, an activity matched control of human elastase was also run.

HNE induced lung haemorrhage in the rat

Instillation of human neutrophil elastase (HNE) into rat lung causes acute lung damage. The extent of this injury can be assessed by measuring lung haemorrhage. Male Sprague Dawley rats (175-220g) were obtained from Harlan UK Ltd., full barrier-bred and certified free from specified micro-organisms on receipt. Animals were weighed and randomly assigned to treatment groups (7-12 animals per group).

The vehicle used was 1% DMSO/Saline. Inhibitors were dissolved in 1% DMSO before the addition of 0.9% saline.

Animals in each study used to determine the efficacy of the elastase inhibitors delivered locally to the lung by a variety of routes. Rats were anaesthetised with the inhaled anaesthetic Isoflurane (4%) when the dose was given from 30 minutes to 6h prior

to human neutrophil elastase (HNE) administration or terminally anaesthetised with hypnorm:hypnovel:water (1.5:1:2 at 2.7ml/kg) when the predose was given at less than 30 minutes prior to HNE administration and dosed either intratracheally (i.t.) by transoral administration using a Penn Century microsprayer or intranasally (i.n.) by dropping the fluid on to the nares. Animals either received vehicle or compound at a dose volume of 0.5ml/kg.

Animals that had been allowed to recover after dosing were terminally anaesthetised with hypnorm:hypnovel:water (1.5:1:2 at 2.7ml/kg). Once sufficiently anaesthetised, HNE (600units/ml) or sterile saline was administered by transoral tracheal instillation at a volume of 100µl using a Penn Century microsprayer. Animals were kept warm in a temperature controlled box and given top up doses of anaesthetic as required to ensure continuous anaesthesia until termination.

Animals were sacrificed (0.5ml to 1ml sodium pentobarbitone) one hour post HNE challenge. The trachea was exposed and a small incision made between two tracheal rings allowing a cannula (10gauge, O.D. 2-10mm, Portex Ltd.) to be inserted approximately 2cm into the trachea towards the lung. This was secured into place with a cotton ligature. The lungs were then lavaged (BAL) three times with fresh 4ml aliquots of heparinised (10units/ml) phosphate buffered saline (PBS). The resultant BALF was kept on ice until it was centrifuged.

The BALF was centrifuged at 1000 r.p.m. for 10 minutes in a centrifuge cooled to between 4 and 10oC. The supernatant was discarded and the cell pellet resuspended in 1ml 0.1% CETAB/PBS to lyse the cells. Cell lysates were frozen until spectrophotometric analysis for blood content could be made. Standards were prepared by making solutions of whole rat blood in 0.1% CETAB/PBS.

Once defrosted 100µl of each lysed cell suspension was placed into a separate well of a 96 well flat bottomed plate. All samples were tested in duplicate and 100µl 0.1% CETAB/PBS was included on the plate as a blank. The OD of the contents of each well was measured at 415nm using a spectramax 250 (Molecular devices).

A standard curve was constructed by measuring the OD (at 415nm) of different concentrations of blood in 0.1% CETAB/PBS (30, 10, 7, 3, 1, 0.3, 0.1µl/ml).

The amount of blood in each experimental sample was calculated by comparison to the standard curve. Data were then analysed as below:

1) The mean OD for duplicates was calculated
2) The value for the blank was subtracted from the value for all other samples
3) Data were assessed to evaluate the normality of distribution.

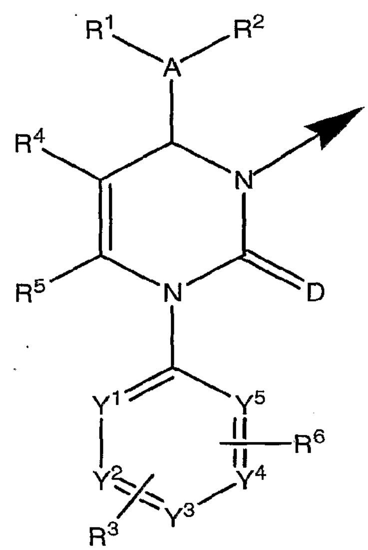
The compounds were shown to have desirable HNE inhibitory activity.

CLAIMS

1. A compound of formula (I)

$$\text{M-L-M} \qquad \text{(I)}$$

wherein L is a linker and each M is independently a group of formula (II):



(II)

wherein A is aryl or heteroaryl;

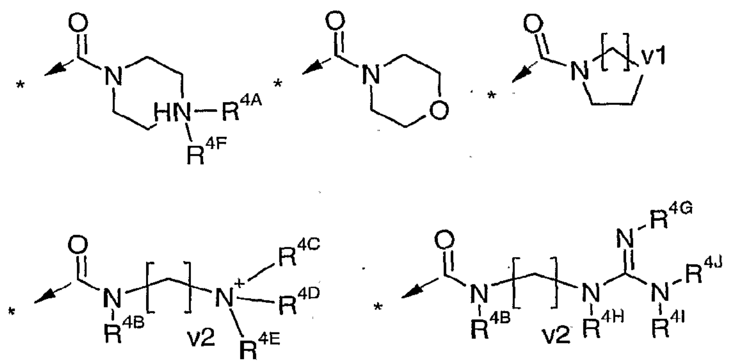
D is oxygen or sulphur;

Y^1, Y^2, Y^3, Y^4 and Y^5 are independently each CH, CR^3, CR^6 or N, with the proviso that one of them is CR^3, one is CR^6 and not more than two are N;

R^1, R^2 and R^3 are independently each hydrogen, halogen, nitro, cyano, C_1-C_6-alkyl, hydroxy or C_1-C_6-alkoxy, wherein alkyl and alkoxy are each optionally substituted with one to three radicals independently selected from halogen, hydroxy and C_1-C_4-alkoxy;

R^4 is trifluoromethylcarbonyl, C_1-C_6-alkylcarbonyl, C_1-C_6-alkoxycarbonyl, C_1-C_6-alkenoxycarbonyl, hydroxycarbonyl, aminocarbonyl, mono- or di-C_1-C_4-alkylaminocarbonyl, C_6-C_{10}-arylaminocarbonyl, arylcarbonyl, heteroarylcarbonyl, heterocyclylcarbonyl, heteroaryl, heterocyclyl or cyano, wherein alkylcarbonyl, alkoxycarbonyl and alkylaminocarbonyl are each optionally substituted with one to three radicals independently selected from C_3-C_8-cycloalkyl, hydroxy, C_1-C_4-alkoxy, C_1-C_4-alkoxycarbonyl, hydroxycarbonyl, aminocarbonyl, mono- and di-C_1-C_4-alkylaminocarbonyl, C_1-C_4-alkylcarbonylamino, (C_1-C_4-alkylcarbonyl)-C_1-C_4-alkylamino, cyano, amino, mono- and di-C_1-C_4-alkylamino, heteroaryl, heterocyclyl and tri-(C_1-C_6-alkyl)-silyl, and wherein heteroarylcarbonyl, heterocyclylcarbonyl, heteroaryl and heterocyclyl can be further substituted with C_1-C_4-alkyl;

or R^4 represents a group of Formula (VIII)



(VIII)

wherein

R^{4A}, R^{4B}, R^{4G}, R^{4H}, R^{4I} and R^{4J} are independently hydrogen or alkyl, or R^{4H} and R^{4I} may may be joined together with the nitrogen atom to which they are attached to form a ring;

R^{4F} is a lone pair or R^{4F} is alkyl and the nitrogen atom to which it is attached is quaternary and carries a positive charge;

R^{4C}, R^{4D} and R^{4E} are alkyl, or any two of R^{4C}, R^{4D} or R^{4E} may be joined together with the nitrogen atom to which they are attached to form a ring, optionally containing a further heteroatom selected from oxygen and nitrogen;

v1 is 1-3; and

v2 is 1-6

R^5 is C_1-C_4-alkyl or amino, and alkyl is optionally substituted with one to three radicals independently selected from halogen, hydroxy, C_1-C_6-alkoxy, C_1-C_6-alkenoxy, C_1-C_6-alkylthio, amino, mono- and di- C_1-C_6-alkylamino, arylamino, hydroxycarbonyl, C_1-C_6-alkoxycarbonyl and the radical -O-(C_1-C_4-alkyl)-O-(C_1-C_4-alkyl); and

R^6 is halogen, nitro, cyano, C_1-C_6-alkyl, hydroxy or C_1-C_6-alkoxy, wherein alkyl and alkoxy are optionally substituted with one to three radicals independently selected from halogen, hydroxy and C_1-C_4-alkoxy;

or a pharmaceutically acceptable salt, solvate or N-oxide thereof.

2. A compound according to claim 1, wherein A is phenyl.

3. A compound according to claim 1 or claim 2, wherein R^1 is hydrogen.

4. A compound according to any preceding claim, wherein R^2 is methyl or -CN.

5. A compound according to any preceding claim, wherein R^3 is hydrogen.

6. A compound according to any preceding claim, wherein R^4 is $-C(O)OCH_2CH_3$ or $-C(O)OCH_2CH_2OH$.

7. A compound according to any preceding claim, wherein R^5 is methyl.

8. A compound according to any preceding claim, wherein R^6 is trifluoromethyl.

9. A compound according to any preceding claim, wherein none of Y^1, Y^2, Y^3, Y^4 and Y^5 is N.

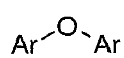
10. A compound according to any preceding claim, wherein D is oxygen.

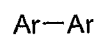
11. A compound according to any preceding claim, which is of the formula (IV)

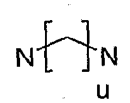
$$(M\text{-}L)_t\text{-}G \qquad (IV)$$

wherein t is 2 to 20; and

G is aryl, heteroaryl, alkyl, cycloalkyl, nitrogen, a dendrimer or a group of any of formulae (V) to (VII):


(V)


(VI)


(VII)

wherein

Ar is aryl or heteroaryl; and

u is 2 to 20.

12. A compound according to claim 11, wherein G is phenoxyphenyl, biphenyl, bipyridyl, ethylenediamino, propylenediamino or a dendrimer.

13. A compound according to any preceding claim, wherein L is a group of formula (III)

$$- L^a - R^7 - L^b - W - L^b - R^7 - L^a - \qquad (III)$$

wherein L^a is a bond or -C(O)-;

L^b is a bond or -C(O)-;

R^7 is alkylene or cycloalkylene;

W is a bond, $N(R^{9B})(R^{9C})$ or is selected from the following divalent radicals

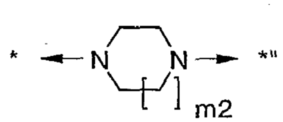
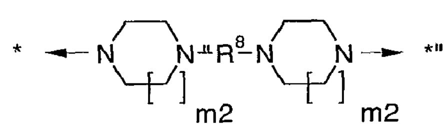
$-(O - R^{8A})_{m1} - O-$

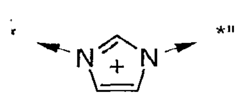
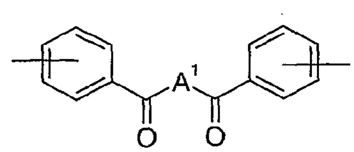
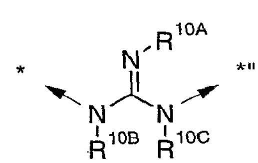
$-N(R^{9A}) - (O - R^{8A})_{m1} - R^{8A} - N(R^{9A})-$

$-N(R^{9A}) - R^{8B} - N(R^{9B})(R^{9C}) - R^{8B} - N(R^{9A})-$

$-N(R^{9A}) - R^{8B} - N(R^{10B})C(=NR^{10A})(NR^{10C}) - R^{8B} - N(R^{9A})-$

$-N(R^{9A}) - R^{8B} - N(R^{9A})-$


;



;

;


wherein

m1 is 1-4;

R^{8A} is alkylene or cycloalkylene;

R^{8B} is an alkylene or cycloalkylene group, or a group of Formula A^2;

R^{9A} is hydrogen or lower alkyl;

one of R^{9B} or R^{9C} is a lone pair and the other is hydrogen or lower alkyl, or R^{9B} and R^{9C} are both lower alkyl, in which case the nitrogen to which they are attached is quaternary and carries a positive charge, or $NR^{9B}R^{9C}$ is a ring;

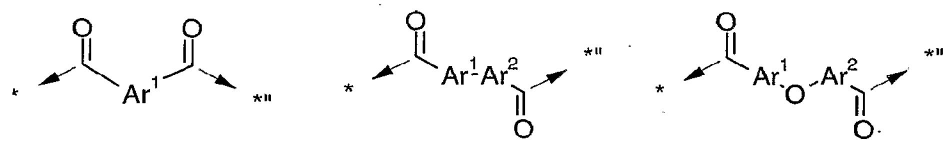
R^{10A} is hydrogen or lower alkyl;

R^{10B} and R^{10C} are independently hydrogen or lower alkyl, or R^{10B} and R^{10C} are joined together to form a ring;

m2 is 1-3;

A^1 is selected from -N(R^{9A})-R^8-N(R^{9B})(R^{9C})-R^8-N(R^{9A})- and -N(R^{9A})-R^8-N(R^{10B})C(=NR^{10A})(NR^{10C})-R^8-N(R^{9A})-; and

A^2 is selected from



wherein Ar^1 and Ar^2 are each independently aryl or heteroaryl.

14. A compound according to claim 13, wherein each L^a is independently -C(O)- or a covalent bond.

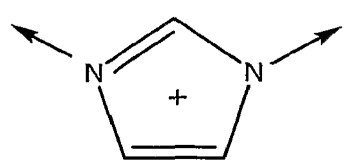
15. A compound according to claim 14, wherein L^a is a bond.

16. A compound according to claims 13 to 15, wherein R^7 is alkylene.

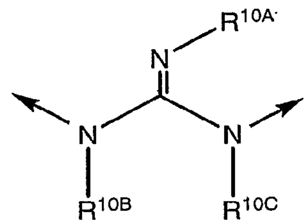
17. A compound according to any of claims 13 to 16, wherein W is -N(R^{9A})-R^{8B}-N(R^{9B})(R^{9C})-R^{8B}-N(R^{9A})-.

18. A compound according to any of claims 13 to 16, wherein W is -N(R^{9A})-R^{8B}-N(R^{10B})C(=NR^{10A})(NR^{10C})-R^{8B}-N(R^{9A}).

19. A compound according to any of claims 13 to 16, wherein W is



20. A compound according to any of claims 13 to 16, wherein W is



21. A compound according to any of claims 13 to 16, wherein W is -N(R^{9B})(R^{9C})-.

22. A compound according to any preceding claim, wherein A is aryl or heteroaryl;
D is oxygen or sulphur;

R^1, R^2 and R^3 are independently each hydrogen, halogen, nitro, cyano, C_1-C_6-alkyl, hydroxy or C_1-C_6-alkoxy, wherein C_1-C_6-alkyl and C_1-C_6-alkoxy can be further substituted with one to three identical or different radicals selected from the group consisting of halogen, hydroxy and C_1-C_4-alkoxy;

R^4 is trifluoromethylcarbonyl, C_1-C_6-alkylcarbonyl, C_1-C_6-alkoxycarbonyl, C_1-C_6-alkenoxycarbonyl, hydroxycarbonyl, aminocarbonyl, mono- or di-C_1-C_4-alkylaminocarbonyl, C_6-C_{10}-arylaminocarbonyl, arylcarbonyl, heteroarylcarbonyl, heterocyclylcarbonyl, heteroaryl, heterocyclyl or cyano, wherein C_1-C_6-alkylcarbonyl, C_1-C_6-alkoxycarbonyl, mono- and di-C_1-C_4-alkylaminocarbonyl can be further substituted with one to three identical or different radicals selected from the group consisting of C_3-C_8-cycloalkyl, hydroxy, C_1-C_4-alkoxy, C_1-C_4-alkoxycarbonyl, hydroxycarbonyl, aminocarbonyl, mono- and di-C_1-C_4-alkylaminocarbonyl, C_1-C_4-alkylcarbonylamino, (C_1-C_4-alkylcarbonyl)-C_1-C_4-alkylamino, cyano, amino, mono- and di-C_1-C_4-alkylamino, heteroaryl, heterocyclyl and tri-(C_1-C_6-alkyl)-silyl, and wherein heteroarylcarbonyl, heterocyclylcarbonyl, heteroaryl and heterocyclyl can be further substituted with C_1-C_4-alkyl;

R^5 is C_1-C_4-alkyl, which can be substituted with one to three identical or different radicals selected from the group consisting of halogen, hydroxy, C_1-C_6-alkoxy, C_1-C_6-alkenoxy, C_1-C_6-alkylthio, amino, mono- and di- C_1-C_6-alkylamino, arylamino, hydroxycarbonyl, C_1-C_6-alkoxycarbonyl and the radical -O-(C_1-C_4-alkyl)-O-(C_1-C_4-alkyl); or R^5 is amino;

R^6 is halogen, nitro, cyano, C_1-C_6-alkyl, hydroxy or C_1-C_6-alkoxy, wherein C_1-C_6-alkyl and C_1-C_6-alkoxy can be further substituted with one to three identical or different radicals selected from the group consisting of halogen, hydroxy and C_1-C_4-alkoxy; and

Y^1, Y^2, Y^3, Y^4 and Y^5 are independently each CH, CR^3, CR^6 or N.

23. A compound according to claim 1, as defined in any of Examples 1 to 76.

24. A compound according to claim 1, as defined in any of Examples 1 to 17.

25. A compound according to claim 1, as defined in any of Examples 22, 24, 33, 35, 36, 38, 41, 46, 65, 69, 70, 71 and 76.

26. A compound according to any preceding claim, for use in therapy.

27. A pharmaceutical composition comprising a compound of any of claims 1 to 25 and a pharmaceutically acceptable carrier or excipient.

28. Use of a compound according to any of claims 1 to 25, for the manufacture of a medicament for use in therapy of a condition selected from asthma, inflammatory bowel diseases, chronic obstructive pulmonary disease (COPD), chronic bronchitis, lung fibrosis, pneumonia, acute respiratory distress syndrome (ARDS), pulmonary emphysema, smoking-induced emphysema, sarcoidosis, bronchiectasis or cystic fibrosis (CF).

29. Use according to claim 28, wherein the condition is COPD.

30. Use according to claim 28, wherein the condition is CF.

31. Use according to claim 28, wherein the condition is ulcerative colitis or Crohn's disease.

32. Use according to any of claims 28 to 31, wherein the condition is respiratory and the medicament is to be administered via the inhaled route.